(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 21 March 2002 (21.03.2002)

(10) International Publication Number

(51) International Patent Classification7:

WO 02/22080 A3

(21) International Application Number:

PCT/US01/28861

C12N 15/86

(22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/233,180

15 September 2000 (15.09.2000)

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- (81) Designated States (national): AE. AG. AL. AM. AT. AU. AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ. DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM. HR. HU, ID, IL. IN. IS. JP. KE. KG. KR, KZ. LC, LK. LR, LS. LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL. TJ. TM, TR. TT. TZ, UA, UG, US, UZ, VN, YU, ZA. ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW). Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT. LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG. CI. CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments
- (88) Date of publication of the international search report: 2 May 2002

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIVI-GAG. POL. NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enm hanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag. HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIVI- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated). HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef. such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



PCT/US01/28861

	SIFICATION OF SUBJECT MATTER : C12N 15/86				
IPC(7) US CL	. 4251456				
according to	International Patent Classification (IPC) or to both na	tional classification and IPC			
	DS SEARCHED				
Minimum doc U.S.: 42	numentation searched (classification system followed b 4/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.3,	y classification symbols) , 235.1, 320.1, 456; 530/23.72;			
Oocumentatio	on searched other than minimum documentation to the	extent that such documents are included	in the fields searched		
Electronic dat Please See Co	ta base consulted during the international search (name ontinuation Sheet	e of data base and, where practicable, s	earch terms used)		
C. DOCU	JMENTS CONSIDERED TO BE RELEVANT				
Category *	Citation of document, with indication, where app	propriate, of the relevant passages	Relevant to claim No.		
X	WO 96/39178 (ERTL et al.) 12 December 1996 (12.	12.1996), see page 5, 6,10, 12, 13	1-3, 8-11, 18		
 Y	and claims 1 and 5.		4, 5, 13-17, 29, 30, 32, 34, 35, 37		
x	US 6,019,978 A (ERTL et al.) 1 February 2000 (01/	/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18		
Υ ,			4, 5, 13-17, 29, 30, 32, 34, 35, 37		
X.P	US 6,287,571 A A (ERTL et al.) 11 September 2001 and claim 1.		1, 9, 18		
X	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/1	997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18		
· Y			4,5,13-17, 29, 30, 32, 34, 35, 37		
Y	WANG et al. The use of an E1-deleted, replication expressing the rabies virus glycoprotein for early va Journal of Virology (March 1997) Vol. 71, No. 5, p	ccination of mice against rables virus.	1-3, 9-11, 13-18		
Furthe	or documents are listed in the continuation of Box C.	See patent family annex.			
*	Special categories of cited documents:	"T" later document published after the int date and not in conflict with the appli principle or theory underlying the inv	cation but cited to understand the		
of partic	ular relevance Application or patent published on or after the international filing date	"X" document of particular relevance; the considered novel or cannot be considered to the consideration of the con	e claimed invention cannot be ered to involve an inventive step		
#I # dommer	nt which may throw doubts on priority claim(s) or which is cited to the publication date of another citation or other special reason (as	"Y" document of particular relevance; the considered to involve an inventive ate combined with one or more other suc	ch documents, such combination		
	document referring to an oral disclosure, use, exhibition or other means being obvious to a person skilled in the art				
priority	priority date claimed				
	actual completion of the international search	Date of mailing of the international se	arch report		
06 February	y 2002 (06.02.2002) mailing address of the ISA/US	Authorized officer	//V		
Co Bo	ommissioner of Patents and Trademarks	Ulrike Winkler, Ph.D.	~ (Jall).). K		
Washington, D.C. 20231 Facsimile No. (703)305-3230 Telephone No. 703-308-0196					
1	SA/210 (second sheet) (July 1998)		A		



INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

zory •	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
Y NA	ATUK et al. Immunogenicity of recombinant human adenovirus -human numunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses 993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29, 30, 32
ad	REVEC et al. Immune response to HIV-1 gag antigens induced by recombinant lenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune eficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29, 30, 32
rei	ORI et al. Rapid protection against human immunodeficiency virus type I (HIV-I) plication mediated by high efficiency non-retroviral delivery of genes interfering with IV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y PI	FARR et al. Differential effects of polyadenylation regions on gene expression in ammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
	ATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) ol. 82, pp. 71-77, see abstract.	1,9
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Inter	l application No.	
PCT/USO	01/28861	

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:
1. Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Claim Nos.: 31 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically: This claim could not be searched because applicant did not provide a CRF.
3. Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet
As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet(1)) (July 1998)



International action No.

PCT/US01/28861

The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
		inserted in E1.
4	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
		inserted in E1.
5	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
	İ	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
		inserted in E1.
6	57-61	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
		to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response
		to HIV Pol protein with the recombinant adenoviral particle in addition to
		administering a DNA plasmid vaccine.
19	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE_1 , the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in the parallel orientation of E1.
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
		inserted in the parallel orientation of E1.
	(7.70.72	The claims are directed to an adenoviral vector that is at least partially deleted of
21	67-70, 72,	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
	73, 75	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in the parallel orientation of E1.
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
22	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
	73, 73	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
20	\ '`	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
		the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
	1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
	- 1	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
	I	the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
		the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
		the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
		inserted in E1.
	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
29	74	The claim is directed to an accitovatal vector date is at least parametry service of

		C.A. wild toma
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
		inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
_		inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
		inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
• •	1 32, 32, 33	to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response
10	05, 5.	to HIV Pol protein with the recombinant adenoviral particle in addition to
		administering a DNA plasmid vaccine.
19	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
17	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
	13, 73	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in the parallel orientation of E1.
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
20		ΔE1, the vector contains the cis-acting packaging sequence of the wild type
	73, 75	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
	1	inserted in the parallel orientation of E1.
	67.70.70	The claims are directed to an adenoviral vector that is at least partially deleted of
21	67-70, 72,	Δ E1, the vector contains the cis-acting packaging sequence of the wild type
	73, 75	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in the parallel orientation of E1.
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22	67-70, 72,	The claims are directed to an adenoviral vector that is at teast partially defected of
	73, 75	ΔΕ1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$.
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
	ì	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
		the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
		the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
	1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
		the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
		the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔEI
2.		and AE3, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
	1	inserted in E1.
20	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
28	74	and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
	}	and ΔE_3 , the vector contains the cis-acting packaging sequence of the which type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
1	- [
L		inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
1		

inserted in E1. The claim is directed to an adenoviral vector that is at least partially deleted of \(\textit{\textit{\textit{\textit{AEI}}}} \) inserted in E1. The claim is directed to an enhold of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein. (SEQ ID NO: 15) inserted in E1. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine. The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed from the claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors. Section 1. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing pol. The claims are drawn to a multivalent vaccine wherein gag and pol are expressed from two individually from one vector. The claims are drawn to a multivalent vaccine wherei			
The claim is directed to an adenoviral vector that is at least partially deleted of \(\textit{\textit{\textit{\textit{AdAE}}}\), the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1. The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine. The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed from three individual vectors. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing gag-pol fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing gag-pol fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing gag-pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing pol. The claims are drawn to a multivalent vaccine wherein gag and pol are expressed individually from one vector. The claims are drawn to a multivalent vaccine wherein pol and nef are expressed from two individually from one vector. The claims are drawn to a multiva			adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
and AE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1. The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed from three individual vectors. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing nef. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing nef. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing nef. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors as a fusion protein. The claims are drawn to a multivalent vaccine wherein gag and pol are expressed individually from one vector. The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually fr		ļ	inserted in E1.
and AE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1. The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle. The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed from three individual vectors. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing nef. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing nef. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing nef. The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors as a fusion protein. The claims are drawn to a multivalent vaccine wherein gag and pol are expressed individually from one vector. The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually fr		74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE :
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The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

REVISED VERSION

(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 21 March 2002 (21.03.2002)

PCT

(10) International Publication Number WO 02/022080 A3

(51) International Patent Classification7:

(21) International Application Number: PCT/US01/28861

(22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

C12N 15/86

(26) Publication Language:

English

(30) Priority Data:

60/233,180

15 September 2000 (15.09.2000)

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD,

Published:

with international search report

(88) Date of publication of the international search report: 2 May 2002 Date of publication of the revised international search report: 16 January 2003

(15) Information about Corrections:

see PCT Gazette No. 03/2003 of 16 January 2003, Section II

Previous Correction:

see PCT Gazette No. 30/2002 of 25 July 2002, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1-Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.



International application No.

PCT/US01/28861

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IPC(7)	SSIFICATION OF SUBJECT MATTER : C12N 15/86			
US CL	: 435/456			
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	UMENTS CONSIDERED TO BE RELEVANT			
Category *	Citation of document, with indication, where ap			Relevant to claim No.
Х	WO 96/39178 (ERTL et al.) 12 December 1996 (12	.12.1996), see page 5,	6,10, 12, 13	1-3, 8-11, 18
	and claims 1 and 5.			
Y				4, 5, 13-17, 29-32, 34, 35, 37
х —	US 6,019,978 A (ERTL et al.) 1 February 2000, (01	/02/2000), see column	s 2, 7 and 8.	1-3, 8-11, 18
Y				4, 5, 13-17, 29-32, 34, 35, 37
X,P	US 6,287,571 8 (ERTL et al.) 11 September 200	1 (11/09/2001), see co	lumns 2, 7, 8	1, 9, 18
x	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/	1997), see examples 1,	2, 25 and 26.	1-3, 8, 9-11, 18
Y				4,5,13-17, 29-32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication expressing the rabies virus glycoprotein for early value of Virology (March 1997) Vol. 71, No. 5, 1	eccination of mice agai		1-3, 9-11, 13-18
5-7				<u> </u>
	documents are listed in the continuation of Box C.	<u> </u>	amily annex.	
* s	pecial categories of cited documents:			nternational filing date or
	t defining the general state of the art which is not considered to ticular relevance			n the application but cited to aderlying the invention
"E" earlier ap	oplication or patent published on or after the international filing	considered no		e claimed invention cannot be dered to involve an inventive ne
	t which may throw doubts on priority claim(s) or which is cited ish the publication date of another citation or other special reason fied)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such		tep when the document is seh documents, such
"O" documen	combination being obvious to a person skilled in the art nent referring to an oral disclosure, use, exhibition or other means "&" document member of the same patent family			
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	nmissioner of Patents and Trademarks PCT	Ulrike Winkler, Ph.	D. NILLE	and the
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Facsimile No	o. (703)305-3230	Telephone No. 703-	308-0196	1/1

Form PCT/ISA/210 (second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US01/28861

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT Category * Citation of document, with indication, where appropriate, of the relevant passages Relevant to claim No. Y NATUK et al. Immunogenicity of recombinant human adenovirus -human 1, 9, 29-32 immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods. Y PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant 1, 9, 29-32 adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract. Y LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) 1,9 replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract. Y PFARR et al. Differential effects of polyadenylation regions on gene expression in 16 mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract. Y NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) 1,9 Vol. 82, pp. 71-77, see abstract.



Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)				
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:				
1. Claim Nos.: because they relate to subject matter not required to be searched by this Authority, namely:				
Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:				
3. Claim Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).				
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)				
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet				
1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims. 2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee. 3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:				
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37				
Remark on Protest The additional search fees were accompanied by the applicant's protest. No protest accompanied the payment of additional search fees.				

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an <u>HIV Pol protein (SEQ ID NO: 1)</u> inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AEI</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔΕ1

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		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
	İ	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1)
		inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
	1	adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
		inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of ΔΕ1
		and ΔE3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7)
		inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant
		adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
		to HIV Pol protein with the recombinant adenoviral particle.
18	63, 64	The claim is directed to a method of generating a cellular mediated immune response
		to HIV Pol protein with the recombinant adenoviral particle in addition to
19	67-70, 72,	administering a DNA plasmid vaccine. The claims are directed to an adenoviral vector that is at least partially deleted of
17	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
	1.2,.2	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in the parallel orientation of E1.
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE_1 , the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
21	67-70, 72,	inserted in the parallel orientation of E1. The claims are directed to an adenoviral vector that is at least partially deleted of
LI	73, 75	ΔE_1 , the vector contains the cis-acting packaging sequence of the wild type
	1.0,.0	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in the parallel orientation of E1.
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	$\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
23	71	inserted in the parallel orientation of E1.
23	1 "	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$, the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
		the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
	İ	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
25	71	the antiparallel orientation of E1. The claim is directed to an adenoviral vector that is at least partially deleted of Δ E1,
2	1"	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
		the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1 ,
	1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
27		the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
	ĺ	inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1
	- (and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
	}	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
20		inserted in E1.
29	74	The claim is directed to an adenoviral vector that is at least partially deleted of ΔE_1
		and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type



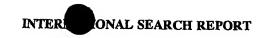
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	T	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing gag-pol fusion and one expressing nef.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing pol.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein <i>pol</i> and <i>nef</i> are expressed as a fusion protein from one vector.
48	860, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as a fusion protein from one vector.

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.



The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be different.

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive concept.

Continuation of B. FIELDS SEARCHED Item 3:

WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

CORRECTED VERSION

(19) World Intellectual Property Organization International Bureau





(43) International Publication Date 21 March 2002 (21.03.2002)

PCT

(10) International Publication Number WO 02/022080 A3

(51) International Patent Classification⁷: C12N 15/86

(21) International Application Number: PCT/US01/28861

(22) International Filing Date:

14 September 2001 (14.09.2001)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

60/233,180

15 September 2000 (15.09.2000) US

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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (84) Designated States (regional): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

- with international search report
- (88) Date of publication of the international search report:

 2 May 2002

 Date of publication of the revised international search report:

 16 January 2003
- (48) Date of publication of this corrected version:

6 March 2003

(15) Information about Corrections:

see PCT Gazette No. 10/2003 of 6 March 2003, Section II Previous Corrections:

see PCT Gazette No. 03/2003 of 16 January 2003, Section II

see PCT Gazette No. 30/2002 of 25 July 2002, Section II

[Continued on next page]

(54) Title: ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

(57) Abstract: First generation adenoviral vectors and associated recombinant adenovirus-based HIV vaccines which show enhanced stability and growth properties and greater cellular-mediated immunity are described within this specification. These adenoviral vectors are utilized to generate and produce through cell culture various adenoviral-based HIV-1 vaccines which contain HIV-1 gag, HIV-1 pol and/or HIV-1 nef polynucleotide pharmaceutical products, and biologically relevant modifications thereof. These adenovirus vaccines, when directly introduced into living vertebrate tissue, preferably a mammalian host such as a human or a non-human mammal of commercial or domestic veterinary importance, express the HIV1- Gag, Pol and/or Nef protein or biologically modification thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding HIV-1 Gag, encoding codon optimized HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The adenoviral vaccines of the present invention, when administered alone or in a combined modality regime, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.







For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

TITLE OF THE INVENTION ENHANCED FIRST GENERATION ADENOVIRUS VACCINES EXPRESSING CODON OPTIMIZED HIV1-GAG, POL, NEF AND MODIFICATIONS

5 CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims the benefit, under 35 U.S.C. §119(e), of U.S. provisional applications 60/233,180, 60/279,056, and Attorney Docket 20867PV2 (serial number unassigned), filed September 15, 2000, March 27, 2001, and September 7, 2001, respectively.

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STATEMENT REGARDING FEDERALLY-SPONSORED R&D Not Applicable

REFERENCE TO MICROFICHE APPENDIX

Not Applicable

FIELD OF THE INVENTION

The present invention relates to recombinant, replication-deficient first generation adenovirus vaccines found to exhibit enhanced growth properties and greater cellular-mediated immunity as compared to other replication-deficient vectors. The invention also relates to the associated first generation adenoviral vectors described herein, which, through the incorporation of additional 5' adenovirus sequence, enhance large scale production efficiency of the recombinant, replicationdefective adenovirus described herein. Another aspect of the instant invention is the surprising discovery that the intron A portion of the human cytomegalovirus (hCMV) promoter constitutes a region of instability in adenoviral vector constructs. Removal of this region from adenoviral expression constructs results in greatly improved vector stability. Therefore, improved vectors expressing a transgene under the control of an intron A-deleted CMV promoter constitute a further aspect of this invention. These adenoviral vectors are useful for generating recombinant adenovirus vaccines against human immunodeficiency virus (HIV). In particular, the first generation adenovirus vectors disclosed herein are utilized to construct and generate adenovirus-based HIV-1 vaccines which contain HIV-1 Gag, HIV-1 Pol and/or HIV-1 Nef polynucleotide pharmaceutical products, and biologically active modifications thereof. Host administration of the recombinant, replication-deficient adenovirus vaccines described herein results in expression of HIV-1 Gag, HIV-1- Pol and/or Nef protein or

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immunologically relevant modifications thereof, inducing a cellular immune response which specifically recognizes HIV-1. The exemplified polynucleotides of the present invention are synthetic DNA molecules encoding codon optimized HIV-1 Gag, HIV-1 Pol, derivatives of optimized HIV-1 Pol (including constructs wherein protease, reverse transcriptase, RNAse H and integrase activity of HIV-1 Pol is inactivated), HIV-1 Nef, and derivatives of optimized HIV-1 Nef, including nef mutants which effect wild type characteristics of Nef, such as myristylation and down regulation of host CD4. The HIV adenovirus vaccines of the present invention, when administered alone or in a combined modality and/or prime/boost regimen, will offer a prophylactic advantage to previously uninfected individuals and/or provide a therapeutic effect by reducing viral load levels within an infected individual, thus prolonging the asymptomatic phase of HIV-1 infection.

BACKGROUND OF THE INVENTION

Human Immunodeficiency Virus-1 (HIV-1) is the etiological agent of acquired human immune deficiency syndrome (AIDS) and related disorders. HIV-1 is an RNA virus of the Retroviridae family and exhibits the 5'LTR-gag-pol-env-LTR 3'organization of all retroviruses. The integrated form of HIV-1, known as the provirus, is approximately 9.8 Kb in length. Each end of the viral genome contains flanking sequences known as long terminal repeats (LTRs). The HIV genes encode at least nine proteins and are divided into three classes; the major structural proteins (Gag, Pol, and Env), the regulatory proteins (Tat and Rev); and the accessory proteins (Vpu, Vpr, Vif and Nef).

The gag gene encodes a 55-kilodalton (kDa) precursor protein (p55) which is expressed from the unspliced viral mRNA and is proteolytically processed by the HIV protease, a product of the pol gene. The mature p55 protein products are p17 (matrix), p24 (capsid), p9 (nucleocapsid) and p6.

The pol gene encodes proteins necessary for virus replication; a reverse transcriptase, a protease, integrase and RNAse H. These viral proteins are expressed as a Gag-Pol fusion protein, a 160 kDa precursor protein which is generated via a ribosomal frame shifting. The viral encoded protease proteolytically cleaves the Pol polypeptide away from the Gag-Pol fusion and further cleaves the Pol polypeptide to the mature proteins which provide protease (Pro, P10), reverse transcriptase (RT, P50), integrase (IN, p31) and RNAse H (RNAse, p15) activities.

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The *nef* gene encodes an early accessory HIV protein (Nef) which has been shown to possess several activities such as down regulating CD4 expression, disturbing T-cell activation and stimulating HIV infectivity.

The env gene encodes the viral envelope glycoprotein that is translated as a 160-kilodalton (kDa) precursor (gp160) and then cleaved by a cellular protease to yield the external 120-kDa envelope glycoprotein (gp120) and the transmembrane 41-kDa envelope glycoprotein (gp41). Gp120 and gp41 remain associated and are displayed on the viral particles and the surface of HIV-infected cells.

The tat gene encodes a long form and a short form of the Tat protein, a RNA binding protein which is a transcriptional transactivator essential for HIV-1 replication.

The rev gene encodes the 13 kDa Rev protein, a RNA binding protein. The Rev protein binds to a region of the viral RNA termed the Rev response element (RRE). The Rev protein promotes transfer of unspliced viral RNA from the nucleus to the cytoplasm. The Rev protein is required for HIV late gene expression and in turn, HIV replication.

Gp120 binds to the CD4/chemokine receptor present on the surface of helper T-lymphocytes, macrophages and other target cells in addition to other co-receptor molecules. X4 (macrophage tropic) virus show tropism for CD4/CXCR4 complexes while a R5 (T-cell line tropic) virus interacts with a CD4/CCR5 receptor complex. After gp120 binds to CD4, gp41 mediates the fusion event responsible for virus entry. The virus fuses with and enters the target cell, followed by reverse transcription of its single stranded RNA genome into the double-stranded DNA via a RNA dependent DNA polymerase. The viral DNA, known as provirus, enters the cell nucleus, where the viral DNA directs the production of new viral RNA within the nucleus, expression of early and late HIV viral proteins, and subsequently the production and cellular release of new virus particles. Recent advances in the ability to detect viral load within the host shows that the primary infection results in an extremely high generation and tissue distribution of the virus, followed by a steady state level of virus (albeit through a continual viral production and turnover during this phase), leading ultimately to another burst of virus load which leads to the onset of clinical AIDS. Productively infected cells have a half life of several days, whereas chronically or latently infected cells have a 3-week half life, followed by non-productively infected cells which have a long half life (over 100 days) but do not significantly contribute to day to day viral loads seen throughout the course of disease.

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Destruction of CD4 helper T lymphocytes, which are critical to immune defense, is a major cause of the progressive immune dysfunction that is the hallmark of HIV infection. The loss of CD4 T-cells seriously impairs the body's ability to fight most invaders, but it has a particularly severe impact on the defenses against viruses, fungi, parasites and certain bacteria, including mycobacteria.

Effective treatment regimens for HIV-1 infected individuals have become available recently. However, these drugs will not have a significant impact on the disease in many parts of the world and they will have a minimal impact in halting the spread of infection within the human population. As is true of many other infectious diseases, a significant epidemiologic impact on the spread of HIV-1 infection will only occur subsequent to the development and introduction of an effective vaccine. There are a number of factors that have contributed to the lack of successful vaccine development to date. As noted above, it is now apparent that in a chronically infected person there exists constant virus production in spite of the presence of anti-HIV-1 humoral and cellular immune responses and destruction of virally infected cells. As in the case of other infectious diseases, the outcome of disease is the result of a balance between the kinetics and the magnitude of the immune response and the pathogen replicative rate and accessibility to the immune response. Pre-existing immunity may be more successful with an acute infection than an evolving immune response can be with an established infection. A second factor is the considerable genetic variability of the virus. Although anti-HIV-1 antibodies exist that can neutralize HIV-1 infectivity in cell culture, these antibodies are generally virus isolate-specific in their activity. It has proven impossible to define serological groupings of HIV-1 using traditional methods. Rather, the virus seems to define a serological "continuum" so that individual neutralizing antibody responses, at best, are effective against only a handful of viral variants. Given this latter observation, it would be useful to identify immunogens and related delivery technologies that are likely to elicit anti-HIV-1 cellular immune responses. It is known that in order to generate CTL responses antigen must be synthesized within or introduced into cells, subsequently processed into small peptides by the proteasome complex, and translocated into the endoplasmic reticulum/Golgi complex secretory pathway for eventual association with major histocompatibility complex (MHC) class I proteins. CD8⁺ T lymphocytes recognize antigen in association with class I MHC via the T cell receptor (TCR) and the CD8 cell surface protein. Activation of naive CD8⁺T cells into activated effector or memory cells generally requires both TCR engagement of antigen as described above as well as engagement of costimulatory proteins. Optimal

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induction of CTL responses usually requires "help" in the form of cytokines from CD4⁺ T lymphocytes which recognize antigen associated with MHC class II molecules via TCR and CD4 engagement.

European Patent Applications 0 638 316 (Published February 15, 1995) and 0 586 076 (Published March 9, 1994), (both assigned to American Home Products Corporation) describe replicating adenovirus vectors carrying an HIV gene, including env or gag. Various treatment regimens were used with chimpanzees and dogs, some of which included booster adenovirus or protein plus alum treatments.

Replication-defective adenoviral vectors harboring deletions in the E1 region are known, and recent adenoviral vectors have incorporated the known packaging repeats into these vectors; e.g., see EP 0 707 071, disclosing, *inter alia*, an adenoviral vector deleted of E1 sequences from base pairs 459 to 3328; and U.S. Patent No. 6,033,908, disclosing, *inter alia*, an adenoviral vector deleted of base pairs 459-3510. The packaging efficiency of adenovirus has been taught to depend on the number of incorporated individual A (packaging) repeats; *see*, *e.g.*, Gräble and Hearing, 1990 *J. Virol.* 66(2):723-731.

Larder, et al., (1987, Nature 327: 716-717) and Larder, et al., (1989, Proc. Natl. Acad. Sci. 86: 4803-4807) disclose site specific mutagenesis of HIV-1 RT and the effect such changes have on in vitro activity and infectivity related to interaction with known inhibitors of RT.

Davies, et al. (1991, *Science* 252:, 88-95) disclose the crystal structure of the RNase H domain of HIV-1 Pol.

Schatz, et al. (1989, FEBS Lett. 257: 311-314) disclose that mutations Glu478Gln and His539Phe in a complete HIV-1 RT/RNase H DNA fragment results in defective RNase activity without effecting RT activity.

Mizrahi, et al. (1990, *Nucl. Acids. Res.* 18: pp. 5359-5353) disclose additional mutations Asp443Asn and Asp498Asn in the RNase region of the *pol* gene which also results in defective RNase activity. The authors note that the Asp498Asn mutant was difficult to characterize due to instability of this mutant protein.

Leavitt, et al. (1993, J. Biol. Chem. 268: 2113-2119) disclose several mutations, including a Asp64Val mutation, which show differing effect on HIV-1 integrase (IN) activity.

Wiskerchen, et al. (1995, J. Virol. 69: 376-386) disclose singe and double mutants, including mutation of aspartic acid residues which effect HIV-1 IN and viral replication functions.

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It would be of great import in the battle against AIDS to produce a prophylactic- and/or therapeutic-based HIV vaccine which generates a strong cellular immune response against an HIV infection. The present invention addresses and meets these needs by disclosing a class of adenovirus vaccines which, upon host administration, express codon optimized and modified versions of the HIV-1 genes, gag, pol and nef. These recombinant, replication-defective adenovirus vaccines may be administered to a host, such as a human, alone or as part of a combined modality regimen and/or prime-boost vaccination regimen with components of the present invention and/or a distinct viral HIV DNA vaccine, non-viral HIV DNA vaccine, HIV subunit vaccine, an HIV whole killed vaccine and/or a live attenuated HIV vaccine.

SUMMARY OF THE INVENTION

The present invention relates to enhanced replication-defective recombinant adenovirus vaccine vectors and associated recombinant, replication-deficient adenovirus vaccines which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef. The adenovirus vaccines of the present invention express HIV antigens and provide for improved cellular-mediated immune responses upon host administration. Potential vaccinees include but are not limited to primates and especially humans and non-human primates, and also include any non-human mammal of commercial or domestic veterinary importance. An effect of the improved recombinant adenovirus-based vaccines of the present invention should be a lower transmission rate to previously uninfected individuals (i.e., prophylactic applications) and/or reduction in the levels of the viral loads within an infected individual (i.e., therapeutic applications), so as to prolong the asymptomatic phase of HIV-1 infection. In particular, the present invention relates to adenoviral-based vaccines which encode various forms of codon optimized HIV-1 Gag (including but in no way limited to p55 versions of codon optimized full length (FL) Gag and tPA-Gag fusion proteins), HIV-1 Pol, HIV-1 Nef, and selected modifications of immunological relevance. The administration, intracellular delivery and expression of these adenovirus vaccines elicit a host CTL and Th response. The preferred replication-defective recombinant adenoviral vaccine vectors include but are not limited to synthetic DNA molecules which (1) encode codon optimized versions of wild type HIV-1 Gag; (2) encode codon optimized versions of HIV-1 Pol; (3) encode codon optimized versions of HIV-1 Pol fusion proteins; (4) encode codon optimized versions of modified HIV-1 Pol proteins and fusion proteins, including but not limited

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to pol modifications involving residues within the catalytic regions responsible for RT, RNase and IN activity within the host cell; (5) encode codon optimized versions of wild type HIV-1 Nef; (6) codon optimized versions of HIV-1 Nef fusion proteins; and/or (7) codon optimized versions of HIV-1 Nef derivatives, including but not limited to nef modifications involving introduction of an amino-terminal leader sequence, removal of an amino-terminal myristylation site and/or introduction of dileucine motif mutations. The Nef-based fusion and modified proteins, disclosed within this specification and expressed from an adenoviral-based vector vaccine this specification, may possess altered trafficking and/or host cell function while retaining the ability to be properly presented to the host MHC I complex and in turn elicit a host CTL and Th response. Examples of HIV-1 Gag, Pol and/or Nef fusion proteins include but are not limited to fusion of a leader or signal peptide at the NH₂-teriminal portion of the viral antigen coding region. Such a leader peptide includes but is not limited to a tPA leader peptide.

The adenoviral vector utilized in construction of the HIV-1 Gag-, HIV-1 Poland/or HIV-1 Nef- based vaccines of the present invention may comprise any replication-defective adenoviral vector which provides for enhanced genetic stability of the recombinant adenoviral genome through large scale production and purification of the recombinant virus. In other words, an HIV-1 Gag-, Pol- or Nef-based adenovirus vaccine of the present invention is a purified recombinant, replicationdefective adenovirus which is shown to be genetically stable through multiple passages in cell culture and remains so during large scale production and purification procedures. Such a recombinant adenovirus vector and harvested adenovirus vaccine lends itself to large scale dose filling and subsequent worldwide distribution procedures which will be demanded of an efficacious monovalent or multivalent HIV vaccine. The present invention meets this basic requirement with description of a replication-defective adenoviral vector and vectors derived therefrom, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome. A preferred embodiment of the instant invention comprises base pairs 1-450 of a wildtype adenovirus. In other preferred embodiments, the replication -defective adenoviral vector has, in addition thereto, a region 3' to the E1-deleted region comprising base pairs 3511-3523. Basepairs 342-450 (more particularly, 400-450) constitute an extension of the 5'region of previously disclosed vectors carrying viral antigens, particularly HIV antigens (see, e.g., PCT International Application PCT/US00/18332, published

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January 11, 2001 (WO 01/02067), which claims priority to U.S. Provisional Application Serial Nos. 60/142,631 and 60/148,981, filed 7/6/1999 and 8/13/1999, respectively; these documents herein incorporated by reference. Applicants have found that extending the 5' region further into the E1 gene into the disclosed vaccine vectors incorporated elements found to be important in optimizing the packaging of the virus.

As compared to previous vectors not comprising basepairs from about 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 of the wildtype adenovirus genome, vectors comprising the above region exhibited enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, a more potent virus effect, allowing lower doses of virus to be used to generate equivalent immunity; and a greater cellular-mediated immune response than replication-deficient vectors not comprising this region (basepairs 1-450). Even more important, adenoviral constructs derived therefrom are very stable genetically in large-scale production, particularly those comprising an expression cassette under the control of a hCMV promoter devoid of intron A. This is because Applicants have surprisingly found that the intron A portion of the hCMV promoter constituted a region of instability when employed in adenoviral vectors. Applicants have, therefore, identified an enhanced adenoviral vector which is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

A preferred embodiment of this invention is a replication-defective adenoviral vector in accordance with the above description wherein the gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

In preferred embodiments, the E1 gene, other than that contained within basepairs 1-450 or, alternatively, that contained within base pairs 1-450 and 3511-3523 has been deleted from the adenoviral vector, and the gene expression cassette has replaced the deleted E1 gene. In other preferred embodiments, the replication defective adenovirus genome does not have a functional E3 gene, or the E3 gene has been deleted. Most preferably, the E3 region is present within the adenoviral genome. Further preferred embodiments are wherein the gene expression cassette is in an E1 anti-parallel (transcribed in a 3' to 5' direction relative to the vector backbone)

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orientation or, more preferably, an E1 parallel (transcribed in a 5' to 3' direction relative to the vector backbone) orientation.

Further embodiments relate to a shuttle plasmid vector comprising: an adenoviral portion and a plasmid portion, wherein said adenovirus portion comprises: a) a replication defective adenovirus genome, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) of the wildtype adenovirus genome and, preferably, in addition thereto, basepairs 3511-3523 of a wildtype adenovirus sequence; and b) a gene expression cassette comprising: (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and (c) a transcription terminator and/or a polyadenylation site.

Other aspects of this invention include a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors.

To this end, the present invention particularly relates to harvested recombinant, replication defective virus derived from a host cell, such as but not limited to 293 cells or PER.C6® cells, including but not limited to harvested virus related to any of the MRKAd5 vector backbones, with or without an accompanying transgene, including but not limited to the HIV-1 antigens described herein. An HIV-1 vaccine is represented by any harvested, recombinant adenovirus material which expresses any one or more of the HIV-1 antigens disclosed herein. This harvested material may then be purified, formulated and stored prior to host administration.

Another aspect of this invention is a method of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising:

a) a recombinant, replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus *cis*-acting adenovirus packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about base pair 458 (preferably, 1-450) and, preferably in addition thereto, base pairs 3511-3523 of a wildtype adenovirus sequence, and,

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b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

In view of the efficacious nature of the adenoviral and/or DNA plasmid vaccines described herein, the present invention relates to all methodology regarding administration of one or more of these adenoviral and/or DNA plasmid vaccines to provide effective immunoprophylaxis, to prevent establishment of an HIV-1 infection following exposure to this virus, or as a post-HIV infection therapeutic vaccine to mitigate the acute HIV-1 infection so as to result in the establishment of a lower virus load with beneficial long term consequences. As discussed herein, such a treatment regimen may include a monovalent or multivalent composition, various combined modality applications, and/or a prime/boost regimen to as to optimize antigen expression and a concomitant cellular-mediated and/or humoral immune response upon inoculation into a living vertebrate tissue. Therefore, the present invention provides for methods of using the adenoviral and/or DNA plasmid vaccines disclosed herein within the various parameters disclosed herein as well as any additional parameters known in the art, which, upon introduction into mammalian tissue induces intracellular expression of the gag, pol and/or nef-based vaccines.

To this end, the present invention relates in part to methods of generating a cellular immune response in a vaccinee, preferably a human vaccinee, wherein the individual is given more than one administration of adenovirus vaccine vector, and it may be given in a regimen accompanied by the administration of a plasmid vaccine. The plasmid vaccine (also referred to herein as a "DNA plasmid vaccine" or "vaccine plasmid" comprises a nucleic acid encoding a protein or an immunologically relevant portion thereof, a heterologous promoter operably linked to the nucleic acid sequence, and a transcription terminator or a polyadenylation signal (such as bGH or SPA, respectively). There may be a predetermined minimum amount of time separating the administrations. The individual can be given a first dose of plasmid vaccine, and then a second dose of plasmid vaccine. Alternatively, the individual may be given a first dose of adenovirus vaccine, and then a second dose of adenovirus vaccine. In other embodiments, the plasmid vaccine is administered first, followed after a time by administration of the adenovirus vaccine. Conversely, the adenovirus vaccine may be administered first, followed by administration of plasmid vaccine after a time. In these embodiments, an individual may be given multiple doses of the same adenovirus serotype in either viral vector or plasmid form, or the virus may be of

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differing serotypes. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

The present invention also relates to multivalent adenovirus vaccine compositions which comprise Gag, Pol and Nef components described herein; see, e.g., Example 29 and Table 25. Such compositions will provide for an enhanced cellular immune response subsequent to host administration, particularly given the genetic diversity of human MHCs and of circulating virus. Examples, but not limitations, include MRKAd5-vector based multivalent vaccine compositions which provide for a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components) composition. Such a mutlivalent vaccine may be filled for a single dose or may consist of multiple inoculations of each individually filled component; and may in addition be part of a prime/boost regimen with viral or non-viral vector vaccines as introduced in the previous paragraph. To this end, preferred compositions are MRKAd5 adenovirus used in combination with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The concept of a "combined modality" as disclosed herein also covers the alternative mode of administration whereby multiple HIV-1 viral antigens may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example, a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possibly a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES). Therefore, a multivalent vaccine delivered as a single, or possibly a second harvested recombinant, replication-deficient adenovirus is contemplated as part of the present invention.

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Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly if pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

It is an object of the present invention to provide for enhanced replication-defective recombinant adenoviral vaccine vector backbones. These recombinant adenoviral backbones may accept one or more transgenes, which may be passaged through cell culture for growth, amplification and harvest.

It is a further object to provide for enhanced replication-defective recombinant adenoviral vaccine vectors which encode various transgenes.

It is also an object of the present invention to provide for a harvested recombinant, replication-deficient adenovirus which shows enhanced growth and amplification rates while in combination with increased virus stability after continuous passage in cell culture. Such a recombinant adenovirus is particularly suited for use in gene therapy and nucleotide-based vaccine vectors which, favorably, lends itself to large scale propagation.

To this end, it is an object of the present invention to provide for (1) enhanced replication-defective recombinant adenoviral vaccine vectors as described herein which encode various forms of HIV-1 Gag, HIV-1 Pol, and/or HIV-1 Nef, including immunologically relevant modifications of HIV-1 Gag, HIV-1 Pol and HIV-1 Nef, and (2) harvested, purified recombinant replication-deficient adenovirus generated by passage of the adenoviral vectors of (1) through one or multiple passages through cell culture, including but not limited to passage through 293 cells or PER.C6® cells.

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It is also an object of the present invention to provide for recombinant adenovirus harvested by one or multiple passages through cell culture. As relating to recombinant adenoviral vaccine vector, this recombinant virus is harvested and formulated for subsequent host administration.

It is also an object of the present invention to provide for replication-defective adenoviral vectors wherein at least one gene is inserted in the form of a gene expression cassette comprising (a) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (b) a heterologous promoter operatively linked to the nucleic acid of part a); and, (c) a transcription terminator.

It is also an object of the present invention to provide for a host cell comprising said adenoviral vectors and/or said shuttle plasmid vectors; vaccine compositions comprising said vectors; and methods of producing the vectors comprising (a) introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and (b) harvesting the resultant adenoviral vectors. It is a further object of the present invention to provide for methods of generating a cellular immune response against a protein in an individual comprising administering to the individual an adenovirus vaccine vector comprising a) a replication defective adenoviral vector, at least partially deleted in E1, comprising a wildtype adenovirus cis-acting packaging region from about base pair 1 to between from about base pair 342 (more preferably, 400) to about 450 (preferably, 1-450) and, preferably, 3511-3523 of a wildtype adenovirus sequence, and, b) a gene expression cassette comprising:(i) a nucleic acid encoding a protein or biologically active and/or immunologically relevant portion thereof; (ii) a heterologous promoter operatively linked to the nucleic acid of part a); and (iii) a transcription terminator and/or a polyadenylation site.

It is also an object of the present invention to provide various alternatives for vaccine administration regimes, namely administration of one or more adenoviral and/or DNA plasmid vaccines described herein to provide effective immunoprophylaxis for uninfected individuals or a therapeutic treatment for HIV infected patients. Such processes include but are not limited to multivalent HIV-1 vaccine compositions, various combined modality regimes as well as various prime/boost alternatives. These methods of administration, relating to vaccine composition and/or scheduled administration, will increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

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As used throughout the specification and claims, the following definitions and abbreviations are used:

"HAART" refers to - highly active antiretroviral therapy -.

"first generation" vectors are characterized as being replication-defective.

They typically have a deleted or inactivated E1 gene region, and preferably have a deleted or inactivated E3 gene region as well.

"AEX" refers to Anion Exchange chromatography.

"QPA" refers to Quick PCR-based Potency Assay.

"bps" refers to basepairs.

"s" or "str" denotes that the transgene is in the E1 parallel or "straight" orientation.

"PBMCs" refers to peripheral blood monocyte cells.

"FL" refers to full length.

"FLgag" refers to a full-length optimized gag gene, as shown in Figure 2.

"Ad5-Flgag" refers to an adenovirus serotype 5 replication deficient virus which carries an expression cassette which comprises a full length optimized gag gene under the control of a CMV promoter.

"Promoter" means a recognition site on a DNA strand to which an RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcriptional activity. The complex can be modified by activating sequences such as enhancers or inhibiting sequences such as silencers.

"Leader" means a DNA sequence at the 5' end of a structural gene which is transcribed along with the gene. This usually results a protein having an N-terminal peptide extension, often referred to as a pro-sequences.

"Intron" means a section of DNA occurring in the middle of a gene which does not code for an amino acid in the gene product. The precursor RNA of the intron is excised and is therefore not transcribed into mRNA not translated into protein.

"Immunologically relevant" or "biologically active" means (1) with regards to a viral protein, that the protein is capable, upon administration, of eliciting a measurable immune response within an individual sufficient to retard the propagation and/or spread of the virus and/or to reduce the viral load present within the individual; or (2) with regards to a nucleotide sequence, that the sequence is capable of encoding for a protein capable of the above.

"Cassette" refers to a nucleic acid sequence which is to be expressed, along with its transcription and translational control sequences. By changing the cassette, a vector can express a different sequence.

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"bGHpA" refers to the bovine growth hormone transcription terminator/polyadenylation sequence.

"tPAgag" refers to a fusion between the leader sequence of the tissue plasminogen activator leader sequence and an optimized HIV gag gene, as exemplified in Figure 30A-B, whether in a DNA or adenovirus-based vaccine vector.

Where utilized, "IA" or "inact" refers to an <u>inactivated</u> version of a gene (e.g. IApol).

"MCS" is "multiple cloning site".

In general, adenoviral constructs, gene constructs are named by reference to the genes contained therein. For example:

"Ad5 HIV-1 gag", also referred to as the original HIV-1 gag adenoviral vector, is a vector containing a transgene cassette composed of a hCMV intron A promoter, the full length version of the human codon-optimized HIV-1 gag gene, and the bovine growth hormone polyadenylation signal. The transgene was inserted in the E1 antiparallel orientation in an E1 and E3 deleted adenovector.

"MRK Ad5 HIV-1 gag" also referred to as "MRKAd5gag" or "Ad5gag2" is an adenoviral vector taught herein which is deleted of E1, comprises basepairs 1-450 and 3511-3523, and has a human codon-optimized HIV-1 gene in an E1 parallel orientation under the control of a CMV promoter without intron A. The construct also comprises a bovine growth hormone polyadenylation signal.

"pV1JnsHIVgag", also referred to as "HIVFLgagPR9901", is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone.

"pV1JnsCMV(no intron)-FLgag-bGHpA" is a plasmid derived from pV1JnsHIVgag which is deleted of the intron A portion of CMV and which comprises the full length HIV gag gene. This plasmid is also referred to as "pV1JnsHIVgag-bGHpA", pV1Jns-hCMV-FL-gag-bGHpA" and "pV1JnsCMV(no intron) + FLgag + bGHpA".

"pV1JnsCMV(no intron)-FLgag-SPA" is a plasmid of the same composition as pV1JnsCMV(no intron)-FLgag-bGHpA except that the SPA termination sequence replaces that of bGHpA. This plasmid is also referred to as "pV1Jns-HIVgag-SPA" and pV1Jns-hCMV-FLgag-SPA".

"pdelE1sp1A" is a universal shuttle vector with no expression cassette (i.e., no promoter or polyA). The vector comprises wildtype adenovirus serotype 5 (Ad5) sequences from bp 1 to bp 341 and bp 3524 to bp 5798, and has a multiple cloning

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site between the Ad5 sequences ending 341 bp and beginning 3524 bp. This plasmid is also referred to as the original Ad 5 shuttle vector.

"MRKpdelE1sp1A" or "MRKpdelE1(Pac/pIX/pack450)" or

"MRKpdelE1(Pac/pIX/pack450)Cla1" is a universal shuttle vector with no expression cassette (i.e. no promoter or polyA) comprising wildtype adenovirus serotype 5 (Ad5) sequences from bp1 to bp450 and bp 3511 to bp 5798. The vector has a multiple cloning site between the Ad5 sequence ending 450 bp and beginning 3511 bp. This shuttle vector may be used to insert the CMV promoter and the bGHpA fragments in both the straight ("str". or E1 parallel) orientation or in the opposite (opp. or E1 antiparallel) orientation)

"MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" is still another shuttle vector which is the modified vector that contains the CMV promoter (no intronA) and the bGHpA fragments. The expression unit containing the hCMV promoter (no intron A) and the bovine growth hormone polyadenylation signal has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BgIII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1/E3+)Cla1 pre-plasmid. This shuttle vector, as shown in Figures 22 and 23, was used to insert the respective IApol and G2A,LLAA nef genes directly into.

"MRKpdelE1-CMV(no intron)-FLgag-bGHpA" is a shuttle comprising Ad5 sequences from basepairs 1-450 and 3511-5798, with an expression cassette containing human CMV without intron A, the full-length human codon-optimized HIV gag gene and bovine growth hormone polyadenylation signal. This plasmid is also referred to as "MRKpdelE1 shuttle +hCMV-FL-gag-BGHpA"

"MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA" is an adenoviral vector comprising all Ad5 sequences except those nucleotides encompassing the E1 region (from 451-3510), a human CMV promoter without intron A, a full-length human codon-optimized HIV gag gene, and a bovine growth hormone polyadenylation signal. This vector is also referred to as "MRKpAdHVE3 + hCMV-FL-gag-BGHpA", "MRKpAd5HIV-1gag", "MRKpAd5gag", "pMRKAd5gag" or "pAd5gag2".

"pV1Jns-HIV-pol inact(opt)" or "pV1Jns-HIV IA pol (opt) is the inactivated Pol gene (contained within SEQ ID NO:3) cloned into the BglII site of V1Jns (Figure 17A-C). As noted herein, various derivatives of HIV-1 pol may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

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"MRKpdel+hCMVmin+FL-pol+bGHpA(s)" is the "MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.)" shuttle mentioned above which contains the IA pol gene is the proper orientation. This shuttle vector is used in a bacterial recombination with MRKpAd(E1-/E3+)Cla1.

"MRKpAd+hCMVmin+FL-pol+bGHpA(S)E3+", also referred to herein as "pMRKAd5pol", is the pre-adenovirus plasmid which comprises a CMV-pol inact(opt)-pGHpA construct. The construction of this pre-adenovirus plasmid is shown in Figure 22.

"pV1Jns/nef (G2A,LLAA)" or "V1Jns/opt nef (G2A,LLAA)" comprises codon optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175 (SEQ ID NO:13; which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662). This fragment is subcloned into the Bgl II site of V1Jns and/orV1Jns-tPA (Figures 16A-B). As noted above for HIV-1 pol, HIV-1 nef constructs may be cloned into a plasmid expression vector such as V1Jns or V1Jns-tPA, thus serving directly as DNA vaccine candidates or as a source for subcloning into an appropriate adenoviral vector.

"MRKpdelE1hCMVminFL-nefBGHpA(s)", also referred to herein as "pMRKAd5nef", is the pre-adenovirus plasmid which comprises a CMV-nef (G2A,LLAA) codon optimized sequence. The construction of this pre-adenovirus plasmid is shown in Figure 23.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the original HTV-1 gag adenovector (Ad5HIV-1gag). This vector is disclosed in PCT International Application No. PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S. Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S. Application Serial No. 60/148,981, filed August 13, 1999, all three applications which are hereby incorporated by reference.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 29) of the optimized human HIV-1 gag open reading frame.

Figure 3 shows diagrammatically the new transgene constructs in comparison with the original gag transgene.

Figure 4 shows the modifications made to the original adenovector backbone in the generation of the novel vectors of the instant invention.

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Figure 5 shows the virus mixing experiments that were carried out to determine the effects of the addition made to the packaging signal region (Expt. #1) and the E3 gene on viral growth (Expt. #2). The bars denote the region of modifications made to the E1 deletion.

Figure 6 shows an autoradiograph of viral DNA analysis following the viral mixing experiments described in Examples 6 and 7.

Figures 7A, 7B and 7C are as follows: Figure 7A shows the hCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7B shows the hCMV-Flgag-SPA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Again, both E1 parallel and E1 antiparallel transgene orientation are represented. Figure 7C shows the mCMV-Flgag-bGHpA adenovectors constructed within the MRKpAdHVE3 and MRKpAdHVO adenovector backbones. Once again, both E1 parallel and E1 antiparallel transgene orientation are represented.

Figure 8A shows the experiment designed to test the effect of transgene orientation.

Figure 8B shows the experiments designed to test the effect of polyadenylation signal.

Figure 9 shows viral DNA from the four adenoviral vectors tested (Example 12) at P5, following *Bst*E11 digestion.

Figure 10 shows viral DNA analysis of passages 11 and 12 of MRKpAdHVE3, MRKAd5HIV-1gag, and MRKAd5HIV-1gagE3-.

Figure 11 shows viral DNA analysis (*Hind*III digestion) of passage 6 MRKpAdHVE3 and MRKAd5HIV-1gag used to initiate the viral competition study. The last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI of 280 viral particles).

Figure 12 shows viral DNA analysis by *Hind* III digestion on high passage numbers for MRKAd5HIV-1gag in serum-containing media with collections made at specified times. The first lane shows the 1kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hind*III), MRKAd5HIV-1gag at P16, P19, and P21.

Figure 13 shows serum anti-p24 levels at 3 wks post i.m. immunization of balb/c mice (n=10) with varying doses of several Adgag constructs: (A) MRK Ad5 HIV-1 gag (through passage 5); (B) MRKAd5 hCMV-FLgag-bGHpA (E3-); (C) MRKAd5 hCMV-FLgag-SPA (E3+); (D) MRKAd5 mCMV-FLgag-bGHpA (E3+);

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(E) research lot (293 cell-derived) of Ad5HIV-1 gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1 gag. Reported are the geometric mean titers (GMT) for each cohort along with the standard error bars.

Figure 14 shows a restriction map of the pMRKAd5HIV-1gag vector.

Figures 15A-X illustrates the nucleotide sequence of the pMRKAd5HIV-1gag vector (SEQ ID NO:27.[coding] and SEQ ID NO:28 [non-coding]).

Figures 16A-B shows a schematic representation of DNA vaccine expression vectors V1Jns (A) and V1Jns-tPA (B), which are utilized for HIV-1 gag, pol and nef constructs in various DNA/viral vector combined modality regimens as disclosed herein.

Figures 17A-C shows the nucleotide (SEQ ID NO:3) and amino acid sequence (SEQ ID NO:4) of IA-Pol. Underlined codons and amino acids denote mutations, as listed in Table 1.

Figure 18 shows codon optimized nucleotide and amino acid sequences through the fusion junction of tPA-pol inact(opt) (contained within SEQ ID NOs: 7 and 8, respectively). The underlined portion represents the NH₂-terminal region of IA-Pol.

Figures 19A-B show a nucleotide sequence comparison between wild type nef(jrfl) and codon optimized nef. The wild type nef gene from the jrfl isolate consists of 648 nucleotides capable of encoding a 216 amino acid polypeptide. WT, wild type sequence (SEQ ID NO:19); opt, codon-optimized sequence (contained within SEQ ID NO:1). The Nef amino acid sequence is shown in one-letter code (SEQ ID NO:2).

Figures 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively. For both versions of the tpanef fusion genes, the putative leader peptide cleavage sites are

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indicated with "*", and a exogenous serine residue introduced during the construction of the mutants is underlined.

Figure 22 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Pol.

Figure 23 shows diagrammatically the construction of the pre-adenovirus plasmid construct, MRKAd5Nef.

Figure 24 shows a comparison of clade B vs. clade C anti-gag T cell responses in clade B HIV-infected subjects.

Figure 25 shows a comparison of clade B vs. clade C anti-nef T cell responses in clade B HIV-infected subjects.

Figures 26A-AO illustrates the nucleotide sequence of the pMRKAd5HIV-1pol adenoviral vector (SEQ ID NO:32 [coding] and SEQ ID NO:33 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO3).

Figures 27A-AM illustrates the nucleotide sequence of the pMRKAd5HIV-1 nef adenoviral vector (SEQ ID NO:34 [coding] and SEQ ID NO:35 [non-coding]), comprising the coding region of the inactivated pol gene (SEQ ID NO13).

Figure 28 shows the stability of MRKAd5 vectors comprising various promoter fragments (hCMV or mCMV) and terminations signals (bGH or SPA) in E3(+) or E3(-) backbones.

Figures 29A and B shows the anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36, 48, and 60 hpi time points (Figure 29A) and the timcourse QPA supernatant titers (Figure 29B) for MRKAd5gag, MRKAd5pol and MRKAd5nef.

Figure 30 shows the nucleotide sequence (SEQ ID NO:36) and amino acid sequence (SEQ ID NO:37) comprising the open reading frame of a representative tPA-gag fusion for use in the DNA and/or adenoviral vaccine disclosed herein.

Figure 31 shows the intracellular γIFN staining of PBMCs collected at week 10 (post DNA prime) and week 30 (post Ad boost). The cells were stimulated overnight in the presence or absence of the gag peptide pool. They were subsequently stained using fluorescence-tagged anti-CD3, anti-CD8, anti-CD4, and anti-γIFN monoclonal antibodies. Each plot shows all CD3+ T cells which were segregated in terms of positive staining for surface CD8 and γIFN production. The numbers in the upper right and lower right quadrants of each plot are the percentages of CD3+ cells that were CD8+γIFN+ and CD4+γIFN+, respectively.

Figure 32 shows a comparison of single-modality adenovirus immunization with DNA + adjuvant prime/adenovirus boost immunization.

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Figures 33A-B show the nucleotide sequence (SEQ ID NO: 38) of the open reading frame for the gag-IApol fusion of Example 29.

Figures 34A-B show the protein sequence (SEQ ID NO:39) of the gag-IApol fustion frame.

DETAILED DESCRIPTION OF THE INVENTION

A novel replication-defective, or "first generation," adenoviral vector suitable for use in gene therapy or nucleotide-based vaccine vectors is described. This vector is at least partially deleted in E1 and comprises a wildtype adenovirus cis-acting packaging region from about base pair 1 to between about base pair 342 (more preferably, 400) to about 458 (preferably, 1-450) and, preferably, 3511-3523 of a wild-type adenovirus sequence. It has been found that a vector of this description possesses enhanced growth characteristics, with approximately 5-10 fold greater amplification rates, and is more potent allowing lower doses of virus to be used to generate equivalent immunity. The vector, furthermore, generates a harvested recombinant adenovirus which shows greater cellular-mediated immune responses than replication-deficient vectors not comprising this region (basepairs 342-450). Adenoviral constructs derived from these vectors are, further, very stable genetically, particularly those comprising a transgene under the control of a hCMV promoter devoid of intron A. Viruses in accordance with this description were passaged continually and analyzed; see Example 12. Each virus analyzed maintained it correct genetic structure. Analysis was also carried out under propagation conditions similar to that performed in large scale production. Again, the vectors were found to possess enhanced genetic stability; see Figure 12. Following 21 passages, the viral DNA showed no evidence of rearrangement, and was highly reproducible from one production lot to the next. The outcome of all relevant tests indicate that the adenoviral vector is extremely well suited for large-scale production of recombinant, replication-deficient adenovirus, as shown herein with the data associated with Figure 28.

A preferred adenoviral vector in accordance with this description is a vector comprising basepairs 1-450, which is deleted in E3. This vector can accommodate up to approximately 7,500 base pairs of foreign DNA inserts (or exogenous genetic material). Another preferred vector is one retaining E3 which comprises basepairs 1-450. A preferred vector of this description is an E3+ vector comprising basepairs 1-450 and 3511-3523. This vector, when deleted of the region spanning basepairs 451-3510, can accommodate up to approximately, 4,850 base pairs of foreign DNA inserts

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(or exogenous genetic material). The cloning capacities of the above vectors have been determined using 105% of the wildtype Ad5 sequence as the upper genome size limit.

Wildtype adenovirus serotype 5 is used as the basis for the specific basepair numbers provided throughout the specification. The wildtype adenovirus serotype 5 sequence is known and described in the art; see, Chroboczek et al., 1992 J. Virology 186:280, which is hereby incorporated by reference. Accordingly, a particular embodiment of the instant invention is a vector based on the adenovirus serotype 5 sequence. One of skill in the art can readily identify the above regions in other adenovirus serotypes (e.g., serotypes 2, 4, 6, 12, 16, 17, 24, 31, 33, and 42), regions defined by basepairs corresponding to the above basepair positions given for adenovirus serotype 5. Accordingly, the instant invention encompasses all adenoviral vectors partially deleted in E1 comprising basepairs corresponding to 1-450 (particularly, 342-450) and, preferably, 3511-3523 of a wild-type adenovirus serotype 5 (Ad5) nucleic acid sequence. Particularly preferred embodiments of the instant invention are those derived from adenoviruses like Ad5 which are classified in subgroup C (e.g., Ad2).

Vectors in accordance with the instant invention are at least partially deleted in E1. Preferably the E1 region is completely deleted or inactivated. Most preferably, the region deleted of E1 is within basepairs 451-3510. It is to be noted that the extended 5' and 3' regions of the disclosed vectors are believed to effectively reduce the size of the E1 deletion of previous constructs without overlapping any part of the E1A/E1B gene present in the cell line used, i.e., the PER.C6® cell line transefected with base pairs 459-3510. Overlap of adenoviral sequences is avoided because of the possibility of recombination. One of ordinary skill in the art can certainly appreciate that the instant invention can, therefore, be modified if a different cell line transfected with a different segment of adenovirus DNA is utilized. For purposes of exemplification, a 5' region of base pairs 1 to up to 449 is more appropriate if a cell line is transfected with adenoviral sequence from base pairs 450-3510. This holds true as well in the consideration of segments 3' to the E1 deletion.

Preferred embodiments of the instant invention possess an intact E3 region (i.e., an E3 gene capable of encoding a functional E3). Alternate embodiments have a partially deleted E3, an inactivated E3 region, or a sequence completely deleted of E3. Applicants have found, in accordance with the instant invention, that virus comprising the E3 gene were able to amplify more rapidly compared with virus not comprising an E3 gene; see Figure 6 wherein a diagnostic CsCl band corresponding to the E3+ virus

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tested (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. These results were obtained following a virus competition study involving mixing equal MOI ratio (1:1) of adenovectors both comprising the E3 gene and not comprising the E3 gene. This increased amplification capacity of the E3+ adenovectors was subsequently confirmed with growth studies; see Table 4A, wherein the E3+ virus exhibit amplification ratios of 470, 420 and 320 as compared with the 115 and 40-50 of the E3- constructs.

As stated above, vectors in accordance with the instant invention can accommodate up to approximately 4,850 base pairs of exogenous genetic material for an E3+ vector and approximately 7,500 base pairs for an E3- vector. Preferably, the insert brings the adenoviral vector as close as possible to a wild-type genomic size (e.g., for Ad5, 35,935 basepairs). It is well known that adenovirus amplifies best when they are close to their wild-type genomic size.

The genetic material can be inserted in an E1-parallel or an E1 anti-parallel orientation, as such is illustrated in Figure 7A, 7B, 7C and Figure 8A. Particularly preferred embodiments of the instant invention, have the insert in an E1-parallel orientation. Applicants have found, via competition experiments with plasmids containing transgenes in differing orientation (Figure 8A), that vector constructs with the foreign DNA insert in an E1-parallel orientation amplify better and actually outcompete E1-antiparallel-oriented transgenes. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation as compared with the E1 anti-parallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested.

Adenoviral vectors in accordance with the instant invention are particularly well suited to effectuate expression of desired proteins, one example of which is an HIV protein, particularly an HIV full length gag protein. Exogenous genetic material encoding a protein of interest can exist in the form of an expression cassette. A gene expression cassette preferably comprises (a) a nucleic acid encoding a protein of interest; (b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and (c) a transcription terminator.

The transcriptional promoter is preferably recognized by an eukaryotic RNA polymerase. In a preferred embodiment, the promoter is a "strong" or "efficient" promoter. An example of a strong promoter is the immediate early human cytomegalovirus promoter (Chapman et al, 1991 *Nucl. Acids Res*19:3979-3986, which is incorporated by reference), preferably without intronic sequences. Most preferred

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for use within the instant adenoviral vector is a human CMV promoter without intronic sequences, like intron A. Applicants have found that intron A, a portion of the human cytomegalovirus promoter (hCMV), constitutes a region of instability for adenoviral vectors. CMV without intron A has been found to effectuate (Examples 1-3) comparable expression capabilities in vitro when driving HIV gag expression and, furthermore, behaved equivalently to intron A-containing constructs in Balb/c mice in vivo with respect to their antibody and T-cell responses at both dosages of plasmid DNA tested (20 µg and 200 µg). Those skilled in the art will appreciate that any of a number of other known promoters, such as the strong immunoglobulin, or other eukaryotic gene promoters may also be used, including the EF1 alpha promoter, the murine CMV promoter, Rous sarcoma virus (RSV) promoter, SV40 early/late promoters and the beta-actin promoter.

In preferred embodiments, the promoter may also comprise a regulatable sequence such as the Tet operator sequence. This would be extremely useful, for example, in cases where the gene products are effecting a result other than that desired and repression is sought.

The combination of the CMV promoter (devoid of the intron A region) with the BGH terminator is particularly preferred although other promoter/terminator combinations in the context of FG adenovirus may also be used.

Other embodiments incorporate a leader or signal peptide into the transgene. A preferred leader is that from the tissue-specific plasminogen activator protein, tPA. Examples include but are not limited to the various tPA-gag, tPA-pol and tPA-nef adenovirus-based vaccines disclosed throughout this specification.

In view of the improved adenovirus vectors described herein, an essential portion of the present invention are adenoviral-based HIV vaccines comprising said adenovirus backbones which may be administered to a mammalian host, preferably a human host, in either a prophylactic or therapeutic setting. The HIV vaccines of the present invention, whether administered alone or in combination regimens with other viral- or non-viral-based DNA vaccines, should elicit potent and broad cellular immune responses against HIV that will either lessen the likelihood of persistent virus infection and/or lead to the establishment of a clinically significant lowered virus load

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subject to HIV infection or in combination with HAART therapy, mitigate the effects of previously established HIV infection (antiviral immunotherapy(ARI)). While any HIV antigen (e.g., gag, pol, nef, gp160, gp41, gp120, tat, rev, etc.) may be utilized in the herein described recombinant adenoviral vectors, preferred embodiments include the codon optimized p55 gag antigen (herein exemplified as MRKAd5gag), pol and nef. Sequences based on different Clades of HIV-1 are suitable for use in the instant invention, most preferred of which are Clade B and Clade C. Particularly preferred embodiments are those sequences (especially, codon-optimized sequences) based on concensus Clade B sequences. Preferred versions of the MRKAd5pol and MRKAd5nef series of adenoviral vaccines will encode modified versions of pol or nef, as discussed herein. Preferred embodiments of the MRKAd5HIV-1 vectors carrying HIV envelope genes and modifications thereof comprise the HIV codon-optimized env sequences of PCT International Applications PCT/US97/02294 and PCT/US97/10517, published August 28, 1997 (WO 97/31115) and December 24, 1997, respectively; both documents of which are hereby incorporated by reference.

A most preferred aspect of the instant invention is the disclosed use of the adenoviral vector described above to effectuate expression of HIV gag. Sequences for many genes of many HIV strains are publicly available in GENBANK and primary, field isolates of HIV are available from the National Institute of Allergy and Infectious Diseases (NIAID) which has contracted with Quality Biological (Gaithersburg, MD) to make these strains available. Strains are also available from the World Health Organization (WHO), Geneva Switzerland. It is preferred that the gag gene be from an HIV-1 strain (CAM-1; Myers et al, eds. "Human Retroviruses and AIDS: 1995, IIA3-IIA19, which is hereby incorporated by reference). This gene closely resembles the consensus amino acid sequence for the clade B (North American/European) sequence. Therefore, it is within the purview of the skilled artisan to choose an appropriate nucleotide sequence which encodes a specific HIV gag antigen, or immunologically relevant portion thereof. As shown in Example 25, a clade B or clade C based p55 gag antigen will potentially be useful on a global scale. As noted herein, the transgene of choice for insertion in to a DNA or MRKAd-based adenoviral vector of the present invention is a codon optimized version of p55 gag. Such a MRKAd5gag adenoviral vector is documented in Example 11 and is at least referred to herein as MRKAd5HIV-1gag. Of course, additional versions are contemplated, including but not limited to modifications such as promoter (e.g., mCMV for hCMV) and/or pA-terminations signal (SPA for bGH) switching, as well as generating MRK Ad5 backbones with or without deletion of the Ad5 E3 gene.

The present invention also relates a series of MRKAd5pol-based adenoviral vaccines which are shown herein to generate cellular immune responses subsequent to administration in mice and non-human primate studies. Several of the MRKAd5pol series are exemplified herein. One such adenoviral vector is referred to as MRKAd5hCMV-inact opt pol(E3+), which comprises the MRKAd5 backbone, the 5 hCMV promoter (no intron A), an inactivated pol transgene, and contains the Ad5 E3 gene in the adenoviral backbone. A second exemplified pre-adenovirus plasmid and concomitant virus is referred to as MRKAd5hCMV-inact opt pol(E3-), which is identical to the former adenoviral vector except that the E3 is deleted. Both constructions contain a codon optimized, inactivated version of HIV-1 Pol, wherein at 10 least the entire coding region is disclosed herein as SEQ ID NO:3 and the expressed protein is shown as SEO ID NO:4 (see also Figure 17A-C and Table 1, which show targeted deletion for inactivated pol. This and other preferred codon optimized versions of HIV Pol as disclosed herein are essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International 15 Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference. As disclosed in the above-mentioned documents, the open reading frame for these codon-optimized HIV-1 Pol-based DNA vaccines are represented by codon optimized DNA molecules encoding codon optimized HIV-1 Pol (e.g. SEQ ID NO:2), codon optimized HIV-1 Pol fused to an 20 amino terminal localized leader sequence (e.g. SEQ ID NO:6), and especially preferable, and exemplified by the MRKAd5-Pol construct in e.g., Example 19, biologically inactivated pol ("inact opt Pol"; e.g., SEQ ID NO:4) which is devoid of significant PR, RT, RNase or IN activity associated with wild type Pol. In addition, a construct related to SEQ ID NO:4 is contemplated which contains a leader peptide at 25 the amino terminal region of the IA Pol protein. A specific construct is ligated within an appropriate DNA plasmid vector containing regulatory regions operatively linked to the respective HIV-1 Pol coding region, with or without a nucleotide sequence encoding a functional leader peptide. To this end, various HIV-1 Pol constructs disclosed herein relate to open reading frames for cloning to the enhanced first 30 generation Ad vectors of the present invention (such a series of MRKAd5pol adenoviral vaccine vectors), including but not limited to wild type Pol (comprising the DNA molecule encoding WT opt Pol, as set forth in SEQ ID NO:2), tPA-opt WTPol, (comprising the DNA molecule encoding tPA Pol, as set forth in SEQ ID NO:6), inact 35 opt Pol (comprising the DNA molecule encoding IA Pol, as set forth in SEQ ID NO:4), and tPA-inact opt Pol, (comprising the DNA molecule encoding tPA-inact opt

Pol, as set forth in SEQ ID NO:8). The pol-based versions of enhanced first generation adenovirus vaccines elicit CTL and Th cellular immune responses upon administration to the host, including primates and especially humans. As noted in the above, an effect of the cellular immune-directed vaccines of the present invention should be a lower transmission rate to previously uninfected individuals and/or reduction in the levels of the viral loads within an infected individual, so as to prolong the asymptomatic phase of HIV-1 infection.

The present invention further relates to a series of MRKAd5nef-based adenoviral vaccines which, similar to HIV gag and pol antigens, generate cellular immune responses subsequent to administration in mice and non-human primate 10 studies. The MRKAd5nef series are exemplified herein by utilizing the improved MRK adenoviral backbone in combination with modified versions of HIV nef. These exemplified MRKAd5nef vectors are as follows: (1) MRKAd5hCMVnef(G2A,LLAA) (E3+), which comprises the improved MRKAd5 backbone, a human CMV promoter an intact Ad5 E3 gene and a modified nef gene: (2) MRKAd5mCMV-15 nef(G2A,LLAA) (E3+), which is the same as (1) above but substituting a murine CMV promoter for a human CMV promoter; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+), which is the same as (2) except that the nef transgene is tpanef(LLAA). Codon optimized versions of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and 20 PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. Particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein 25 is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH2-terminus of the HIV-1 Nef 30 polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and 35 substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein

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described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. MRKAd5nef vectors (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) and (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) contain this transgene. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16. The MRKAd5nef vector "MRKAd5mCMV-tpanef(LLAA) (E3+)" contains this transgene.

Along with the improved MRKAd5gag adenovirus vaccine vector described herein, generation of a MRKAd5pol and MRKAd5nef adenovirus vector provide for enhanced HIV vaccine capabilities. Namely, the generation of this trio of adenoviral vaccine vectors, all shown to generate effective cellular immune responses subsequent to host administration, provide for the ability to administer these vaccine candidates not only alone, but preferably as part of a divalent (i.e., gag and nef, gag and pol, or pol and nef components) or a trivalent vaccine (i.e., gag, pol and nef components). Therefore, a preferred aspect of the present invention are vaccine formulations and associated methods of administration and concomitant generation of host cellular immune responses associated with formulating three separate series of MRKAd5based adenoviral vector vaccines. Of course, this MRKAd5 vaccine series based on distinct HIV antigens promotes expanded opportunities for formulation of a divalent or trivalent vaccine, or possibly administration of separate formulations of one or more monovalent or divalent formulations within a reasonable window of time. It is also within the scope of the present invention to embark on combined modality regimes which include multiple but distinct components from a specific antigen. An example, but certainly not a limitation, would be separate MRKAd5pol vectors, with one vaccine vector expressing wild type Pol (SEQ ID NO:2) and another MRKAd5pol vector expressing inactivated Pol (SEQ ID NO:6). Another example might be separate MRKAd5nef vectors, with one vaccine vector expressing the tPA/LLAA version of Nef (SEQ ID NO:16) and another MRKAd5nef vector expressing the G2A,LLAA modified version of Nef (SEQ ID NO:14). Therefore, the MRKAd5 adenoviral vectors of the present invention may be used in combination

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with multiple, distinct HIV antigen classes. Each HIV antigen class is subject to sequence manipulation, thus providing for a multitude of potential vaccine combinations; and such combinations are within the scope of the present invention. The utilization of such combined modalities vaccine formulation and administration increase the probability of eliciting an even more potent cellular immune response when compared to inoculation with a single modality regimen.

The present invention also relates to application of a mono-, dual-, or trimodality administration regime of the MRKAd5gag, pol and nef adenoviral vaccine series in a prime/boost vaccination schedule. This prime/boost schedule may include any reasonable combination of the MRKAd5gag, pol and nef adenoviral vaccine series disclosed herein. In addition, a prime/boost regime may also involve other viral and/or non-viral DNA vaccines. A preferable addition to an adenoviral vaccine vector regime includes but is not limited to plasmid DNA vaccines, especially DNA plasmid vaccines that contain at least one of the codon optimized gag, pol and nef constructions, as disclosed herein.

Therefore, one aspect of this invention is the administration of the adenoviral vector containing the optimized gag gene in a prime/boost regiment in conjunction with a plasmid DNA encoding gag. To distinguish this plasmid from the adenoviralcontaining shuttle plasmids used in the construction of an adenovirus vector, this plasmid will be referred to as a "vaccine plasmid" or "DNA plasmid vaccine". Preferred vaccine plasmids for use in this administration protocol are disclosed in pending U.S. patent application 09/017,981, filed February 3, 1998 and WO98/34640, published August 13, 1998, both of which are hereby incorporated by reference. Briefly, the preferred vaccine plasmid is designated V1Jns-FLgag, which expresses the same codon-optimized gag gene as the adenoviral vectors of this invention (see Figure 2 for the nucleotide sequence of the exemplified optimized codon version of full length p55 gag). The vaccine plasmid backbone, designated V1Jns contains the CMV immediate-early (IE) promoter and intron A, a bovine growth hormone-derived polyadenylation and transcription termination sequence as the gene expression regulatory elements, and a minimal pUC backbone; see Montgomery et al., 1993, DNA Cell Biol. 12:777-783. The pUC sequence permits high levels of plasmid production in E. coli and has a neomycin resistance gene in place of an ampicillin resistance gene to provide selected growth in the presence of kanamycin. Alternatively, a vaccine plasmid which has the CMV promoter deleted of intron A can be used. Those of skill in the art will recognize that alternative vaccine plasmid

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vectors may be easily substituted for these specific constructs, and this invention specifically envisions use of such alternative plasmid DNA vaccine vectors.

Another aspect of the present invention is a prime/boost regimen which includes a vaccine plasmid which encodes an HIV pol antigen, preferably a codon optimized form of pol and also preferably a vaccine plasmid which comprises a nucleotide sequence which encodes a Pol antigen selected from the group of Pol antigens as shown in SEQ ID NOs: 2, 4, 6 and 8. The variety of potential DNA plasmid vaccines which encode various biologically active forms of HIV-1 Pol, wherein administration, intracellular delivery and expression of the HIV-1 Pol gene of interest elicits a host CTL and Th response. The preferred synthetic DNA molecules of the present invention encode codon optimized wild type Pol (without Pro activity) and various codon optimized inactivated HIV-1 Pol proteins. The HIV-1 pol open reading disclosed herein are especially preferred for pharmaceutical uses, especially for human administration as delivered via a recombinant adenoviral vaccine, especially an enhanced first generation recombinant adenoviral vaccine as described herein. Several embodiments of this portion of the invention are provided in detail below, namely DNA molecules which comprise a HIV-1 pol open reading frame, whether encoding full length pol or a modification or fusion as described herein, wherein the codon usage has been optimized for expression in a mammal, especially a human. Again, these DNA sequences are positioned appropriately within a recombinant adenoviral vector, such as the exemplified recombinant adenoviral vector described herein, so as to promote expression of the respective HIV-1 Pol gene of interest, and subsequent to administration, elicit a host CTL and Th response. Again, these preferred, but in no way limiting, pol genes are as disclosed herein and essentially as described in U.S. Application Serial No. 09/745,221, filed December 21, 2000 and PCT International Application PCT/US00/34724, also filed December 21, 2000, both documents which are hereby incorporated by reference.

A third series of vaccine plasmids which are useful in a combined modality and/or prime/boost regimen are vaccine plasmids which encode an HIV nef antigen or biologically and/or immunologically relevant modification thereof. As noted elsewhere, preferred vaccine plasmids contain a codon optimized form of nef and also preferably comprise a nucleotide sequence which encodes a Nef antigen selected from the group of Nef antigens as shown in SEQ ID NOs: 10, 12, 14 and 16. These preferred nef coding regions are disclosed herein, as well as being described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International

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Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference.

Therefore, the adenoviral vaccines and plasmid DNA vaccines of this invention may be administered alone, or may be part of a prime and boost administration regimen. A mixed modality priming and booster inoculation scheme will result in an enhanced immune response, particularly is pre-existing anti-vector immune responses are present. This one aspect of this invention is a method of priming a subject with the plasmid vaccine by administering the plasmid vaccine at least one time, allowing a predetermined length of time to pass, and then boosting by administering the adenoviral vaccine. Multiple primings typically, 1-4, are usually employed, although more may be used. The length of time between priming and boost may typically vary from about four months to a year, but other time frames may be used. In experiments with rhesus monkeys, the animals were primed four times with plasmid vaccines, then were boosted 4 months later with the adenoviral vaccine. Their cellular immune response was notably higher than that of animals which had only received adenoviral vaccine. The use of a priming regimen may be particularly preferred in situations where a person has a pre-existing anti-adenovirus immune response.

Furthermore and in the alternative, multiple HTV-1 viral antigens, such as the MRKAd5 adenoviral vaccines disclosed herein, may be ligated into a proper shuttle plasmid for generation of a pre-adenoviral plasmid comprising multiple open reading frames. For example a trivalent vector may comprise a gag-pol-nef fusion, in either a E3(-) or E3(+) background, preferably a E3 deleted backbone, or possible a "2+1" divalent vaccine, such as a gag-pol fusion (i.e., codon optimized p55 gag and inactivated optimized pol; Example 29 and Table 25) within the same MRKAd5 backbone, with each open reading frame being operatively linked to a distinct promoter and transcription termination sequence. Alternatively, the two open reading frames may be operatively linked to a single promoter, with the open reading frames operatively linked by an internal ribosome entry sequence (IRES), as disclosed in International Publication No. WO 95/24485, which is hereby incorporated by reference. Figure 9 shows that the use of multiple promoters and termination sequences provide for similar growth properties, while Figure 28 shows that these MRKAd5gag-based vectors are also stable at least through passage 21. In the absence of the use of IRES-based technology, it is preferred that a distinct promoter be used to support each respective open reading frame, so as to best preserve vector stability. As examples, and certainly not as limitations, potential multiple transgene vaccines may

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include a three transgene vector such as hCMV-gagpol-bGHpA + mCMV-nef-SPA in an E3 deleted backbone or hCMV-gagpol-bGHpA + mCMV-nef-SPA(E3+). Potential "2+1" divalent vaccines of the present invention might be a hCMV-gagbGHpA + mCMV-nef-SPA in an E3+ backbone (vector #1) in combination with hCMV-pol-bGHpA in an E3+ backbone (vector #2), with all transgenes in the E1 parallel orientation. Fusion constructs other than the gag-pol fusion described above are also suitable for use in various divalent vaccine strategies and can be composed of any two HIV antigens fused to one another (e.g.,, nef-pol and gag-nef). These adenoviral compositions are, as above, preferably delivered along with an adenoviral composition comprising an additional HTV antigen in order to diversify the immune response generated upon administration. Therefore, a multivalent vaccine delivered in a single, or possible second, adenoviral vector is certainly contemplated as part of the present invention. Again, this mode of administration is another example of whereby an efficaceous adenovirus-based HIV-1 vaccine may be administered via a combined modality regime. It is important to note, however, that in terms of deciding on an insert for the disclosed adenoviral vectors, due consideration must be dedicated to the effective packaging limitations of the adenovirus vehicle. Adenovirus has been shown to exhibit an upper cloning capacity limit of approximately 105% of the wildtype Ad5 sequence.

Regardless of the gene chosen for expression, it is preferred that the sequence be "optimized" for expression in a human cellular environment. A "triplet" codon of four possible nucleotide bases can exist in 64 variant forms. That these forms provide the message for only 20 different amino acids (as well as transcription initiation and termination) means that some amino acids can be coded for by more than one codon. Indeed, some amino acids have as many as six "redundant", alternative codons while some others have a single, required codon. For reasons not completely understood, alternative codons are not at all uniformly present in the endogenous DNA of differing types of cells and there appears to exist variable natural hierarchy or "preference" for certain codons in certain types of cells. As one example, the amino acid leucine is specified by any of six DNA codons including CTA, CTC, CTG, CTT, TTA, and TTG (which correspond, respectively, to the mRNA codons, CUA, CUC, CUG, CUU, UUA and UUG). Exhaustive analysis of genome codon frequencies for microorganisms has revealed endogenous DNA of E. coli most commonly contains the CTG leucine-specifying codon, while the DNA of yeasts and slime molds most commonly includes a TTA leucine-specifying codon. In view of this hierarchy, it is generally held that the likelihood of obtaining high levels of expression of a leucine-

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rich polypeptide by an *E. coli* host will depend to some extent on the frequency of codon use. For example, a gene rich in TTA codons will in all probability be poorly expressed in *E. coli*, whereas a CTG rich gene will probably highly express the polypeptide. Similarly, when yeast cells are the projected transformation host cells for expression of a leucine-rich polypeptide, a preferred codon for use in an inserted DNA would be TTA.

The implications of codon preference phenomena on recombinant DNA techniques are manifest, and the phenomenon may serve to explain many prior failures to achieve high expression levels of exogenous genes in successfully transformed host organisms—a less "preferred" codon may be repeatedly present in the inserted gene and the host cell machinery for expression may not operate as efficiently. This phenomenon suggests that synthetic genes which have been designed to include a projected host cell's preferred codons provide a preferred form of foreign genetic material for practice of recombinant DNA techniques. Thus, one aspect of this invention is an adenovirus vector or adenovirus vector in some combination with a vaccine plasmid where both specifically include a gene which is codon optimized for expression in a human cellular environment. As noted herein, a preferred gene for use in the instant invention is a codon-optimized HIV gene and, particularly, HIV gag, pol or nef.

Adenoviral vectors in accordance with the instant invention can be constructed using known techniques, such as those reviewed in Hitt et al, 1997 "Human Adenovirus Vectors for Gene Transfer into Mammalian Cells" Advances in Pharmacology 40:137-206, which is hereby incorporated by reference.

In constructing the adenoviral vectors of this invention, it is often convenient to insert them into a plasmid or shuttle vector. These techniques are known and described in Hitt et al., *supra*. This invention specifically includes both the adenovirus and the adenovirus when inserted into a shuttle plasmid.

Preferred shuttle vectors contain an adenoviral portion and a plasmid portion. The adenoviral portion is essentially the same as the adenovirus vector discussed supra, containing adenoviral sequences (with non-functional or deleted E1 and E3 regions) and the gene expression cassette, flanked by convenient restriction sites. The plasmid portion of the shuttle vector often contains an antibiotic resistance marker under transcriptional control of a prokaryotic promoter so that expression of the antibiotic does not occur in eukaryotic cells. Ampicillin resistance genes, neomycin resistance genes and other pharmaceutically acceptable antibiotic resistance markers may be used. To aid in the high level production of the polynucleotide by

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fermentation in prokaryotic organisms, it is advantageous for the shuttle vector to contain a prokaryotic origin of replication and be of high copy number. A number of commercially available prokaryotic cloning vectors provide these benefits. It is desirable to remove non-essential DNA sequences. It is also desirable that the vectors not be able to replicate in eukaryotic cells. This minimizes the risk of integration of polynucleotide vaccine sequences into the recipients' genome. Tissue-specific promoters or enhancers may be used whenever it is desirable to limit expression of the polynucleotide to a particular tissue type.

In one embodiment of this invention, the pre-plasmids (e.g., pMRKAd5pol, pMRKAd5nef and pMRKAd5gag were generated by homologous recombination using the MRKHVE3 (and MRKHVO for the E3- version) backbones and the appropriate shuttle vector, as shown for pMRKAd5pol in Figure 22 and for pMRKAd5nef in Figure 23. The plasmid in linear form is capable of replication after entering the PER.C6[®] cells and virus is produced. The infected cells and media were harvested after viral replication was complete.

Viral vectors can be propagated in various E1 complementing cell lines, including the known cell lines 293 and PER.C6[®]. Both these cell lines express the adenoviral E1 gene product. PER.C6[®] is described in WO 97/00326 (published January 3, 1997) and issued U.S. Patent No. 6,033,908, both of which are hereby incorporated by reference. It is a primary human retinoblast cell line transduced with an E1 gene segment that complements the production of replication deficient (FG) adenovirus, but is designed to prevent generation of replication competent adenovirus by homologous recombination. Cells of particular interest have been stably transformed with a transgene that encodes the AD5E1A and E1B gene, like PER.C6[®], from 459 bp to 3510 bp inclusive. 293 cells are described in Graham et al., 1977 J. Gen. Virol 36:59-72, which is hereby incorporated by reference. As stated above, consideration must be given to the adenoviral sequences present in the complementing cell line used. It is important that the sequences not overlap with that present in the vector if the possibility of recombination is to be minimized.

It has been found that vectors generated in accordance with the above description are more effective in inducing an immune response and, thus, constitute very promising vaccine candidates. More particularly, it has been found that first generation adenoviral vectors in accordance with the above description carrying a codon-optimized HIV gag gene, regulated with a strong heterologous promoter can be used as human anti-HIV vaccines, and are capable of inducing immune responses.

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Standard techniques of molecular biology for preparing and purifying DNA constructs enable the preparation of the DNA immunogens of this invention.

A vaccine composition comprising an adenoviral vector in accordance with the instant invention may contain physiologically acceptable components, such as buffer, normal saline or phosphate buffered saline, sucrose, other salts and polysorbate. One preferred formulation has: 2.5-10 mM TRIS buffer, preferably about 5 mM TRIS buffer; 25-100 mM NaCl, preferably about 75 mM NaCl; 2.5-10% sucrose, preferably about 5% sucrose; 0.01 -2 mM MgCl₂; and 0.001%-0.01% polysorbate 80 (plant derived). The pH should range from about 7.0-9.0, preferably about 8.0. One skilled in the art will appreciate that other conventional vaccine excipients may also be used it make the formulation. The preferred formulation contains 5mM TRIS, 75 mM NaCl, 5% sucrose, 1mM MgCl₂, 0.005% polysorbate 80 at pH 8.0 This has a pH and divalent cation composition which is near the optimum for Ad5 stability and minimizes the potential for adsorption of virus to a glass surface. It does not cause tissue irritation upon intramuscular injection. It is preferably frozen until use.

The amount of adenoviral particles in the vaccine composition to be introduced into a vaccine recipient will depend on the strength of the transcriptional and translational promoters used and on the immunogenicity of the expressed gene product. In general, an immunologically or prophylactically effective dose of 1×10^{7} to 1×10^{12} particles and preferably about 1×10^{10} to 1×10^{11} particles is administered directly into muscle tissue. Subcutaneous injection, intradermal introduction, impression through the skin, and other modes of administration such as intraperitoneal, intravenous, or inhalation delivery are also contemplated. It is also contemplated that booster vaccinations are to be provided. Following vaccination with HIV adenoviral vector, boosting with a subsequent HIV adenoviral vector and/or plasmid may be desirable. Parenteral administration, such as intravenous, intramuscular, subcutaneous or other means of administration of interleukin-12 protein, concurrently with or subsequent to parenteral introduction of the vaccine compositions of this invention is also advantageous.

The adenoviral vector and/or vaccine plasmids of this invention polynucleotide may be unassociated with any proteins, adjuvants or other agents which impact on the recipients' immune system. In this case, it is desirable for the vector to be in a physiologically acceptable solution, such as, but not limited to, sterile saline or sterile buffered saline. Alternatively, the vector may be associated with an adjuvant known in the art to boost immune responses (i.e., a "biologically effective"

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adjuvant), such as a protein or other carrier. Vaccine plasmids of this invention may, for instance, be delivered in saline (e.g., PBS) with or without an adjuvant. Preferred adjuvants are Alum or CRL1005 Block Copolymer. Agents which assist in the cellular uptake of DNA, such as, but not limited to, calcium ions, may also be used to advantage. These agents are generally referred to herein as transfection facilitating reagents and pharmaceutically acceptable carriers. Techniques for coating microprojectiles coated with polynucleotide are known in the art and are also useful in connection with this invention.

This invention also includes a prime and boost regimen wherein a first adenoviral vector is administered, then a booster dose is given. The booster dose may be repeated at selected time intervals. Alternatively, a preferred inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype. More preferably, the inoculation scheme comprises priming with a first adenovirus serotype and then boosting with a second adenovirus serotype, wherein the first and second adenovirus serotypes are classified within separate subgroups of adenoviruses. The above prime/boost schemes are particularly preferred in those situations where a preexisting immunity is identified to the adenoviral vector of choice. In this type of scheme, the individual or population of individuals is primed with an adenovirus of a serotype other than that to which the preexisting immunity is identified. This enables the first adenovirus to effectuate sufficient expression of the transgene while evading existing immunity to the second adenovirus (the boosting adenovirus) and, further, allows for the subsequent delivery of the transgene via the boosting adenovirus to be more effective. Adenovirus serotype 5 is one example of a virus to which such a scheme might be desirable. In accordance with this invention, therefore, one might decide to prime with a non-group C adenovirus (e.g., Ad12, a group A adenovirus, Ad24, a group D adenovirus, or Ad35, a group B adenovirus) to evade anti-Ad5 immunity and then boost with Ad5, a group C adenovirus. Another preferred embodiment involves administration of a different adenovirus (including non-human adenovirus) vaccine followed by administration of the adenoviral vaccines disclosed. In the alternative, a viral antigen of interest can be first delivered via a viral vaccine other than an adenovirus-based vaccine, and then followed with the adenoviral vaccine disclosed. Alternative viral vaccines include but are not limited to pox virus and venezuelan equine encephilitis virus.

A large body of human and animal data supports the importance of cellular immune responses, especially CTL in controlling (or eliminating) HIV infection. In humans, very high levels of CTL develop following primary infection and correlate

with the control of viremia. Several small groups of individuals have been described who are repeatedly exposed to HIV by remain uninfected; CTL has been noted in several of these cohorts. In the SIV model of HIV infection, CTL similarly develops following primary infection, and it has been demonstrated that addition of anti-CD8 monoclonal antibody abrogated this control of infection and leads to disease progression. This invention uses adenoviral vaccines alone or in combination with plasmid vaccines to induce CTL.

The following non-limiting Examples are presented to better illustrate the invention.

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EXAMPLE 1

Removal of the Intron A Portion of the hCMV Promoter GMP grade pVIInsHIVgag was used as the starting material to amplify the hCMV promoter. PVIInsHIVgag is a plasmid comprising the CMV immediate-early (IE) promoter and intron A, a full-length codon-optimized HIV gag gene, a bovine growth hormone-derived polyadenylation and transcriptional termination sequence, and a minimal pUC backbone; see Montgomery et al., supra for a description of the plasmid backbone. The amplification was performed with primers suitably positioned to flank the hCMV promoter. A 5' primer was placed upstream of the Msc1 site of the hCMV promoter and a 3' primer (designed to contain the BgIII recognition sequence) was placed 3' of the hCMV promoter. The resulting PCR product (using high fidelity Taq polymerase) which encompassed the entire hCMV promoter (minus intron A) was cloned into TOPO PCR blunt vector and then removed by double digestion with Msc1 and BgIII. This fragment was then cloned back into the original GMP grade pV1JnsHIVgag plasmid from which the original promoter, intron A, and the gag gene were removed following Msc1 and BgIII digestion. This ligation reaction resulted in the construction of a hCMV promoter (minus intron A) + bGHpA expression cassette within the original pV1JnsHIVgag vector backbone. This vector is designated pVIJnsCMV(no intron).

The FLgag gene was excised from pV1JnsHIVgag using BgIII digestion and the 1,526 bp gene was gel purified and cloned into pV1JnsCMV(no intron) at the BgIII site. Colonies were screened using Sma1 restriction enzymes to identify clones that carried the Flgag gene in the correct orientation. This plasmid, designated pV1JnsCMV(no intron)-FLgag-bGHpA, was fully sequenced to confirm sequence integrity.

Two additional transgenes were also constructed. The plasmid, pV1JnsCMV(no intron)-FLgag-SPA, is identical to pV1JnsCMV(no intron)-FLgag-bGHpA except that the bovine growth hormone polyadenylation signal has been replaced with a short synthetic polyA signal (SPA) of 50 nucleotides in length. The sequence of the SPA is as shown, with the essential components (poly(A) site, (GT)_n, and (T)_n; respectively) underlined:

<u>AATAAA</u>AGATCTTTATTTTCATTAGATCT<u>GTGTG TTGGTTTTTTGTGTG</u> (SEQ ID NO:18).

The plasmid, pV1Jns-mCMV-FLgag-bGHpA, is identical to the pV1JnsCMV(no intron)-FLgag-bGHpA except that the hCMV promoter has been removed and replaced with the murine CMV (mCMV) promoter.

Figure 3 diagrammatically shows the new transgene constructs in comparison with the original transgene.

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EXAMPLE 2

Gag Expression Assay for Modified Gag Transgenes

Gag Elisa was performed on culture supernatants obtained from transient tissue culture transfection experiments in which the two new hCMV-containing plasmid constructs, pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no intron)-FLgag-SPA, both devoid of intron A, were compared to pV1JnsHIVgag which, as noted above possesses the intron A as part of the hCMV promoter. Table 2 below shows the *in vitro* gag expression data of the new gag plasmids compared with the GMP grade original plasmid. The results displayed in Table 2 show that both of the new hCMV gag plasmid constructs have expression capacities comparable to the original plasmid construct which contains the intron A portion of the hCMV promoter.

Table 2: In vitro DNA transfection of original and new plasmid HIV-1 gag constructs.

Plasmid	μg gag/10e6 COS cells/5μg DNA/48 hr
HIVFL-gagPR9901a	10.8
PVIIns-hCMV-FLgag-bGHpAb	16.6
pV1Jns-hCMV-FLgag-SPA ^{b.c}	12.0

^a GMP grade pV1Jns-hCMVintronA-FLgag-bGHpA.

10 EXAMPLE 3

Rodent (Balb/c) Study for Modified gag Transgenes
A rodent study was performed on the two new plasmid constructs
described above – pV1JnsCMV(no intron)-FLgag-bGHpA and pV1JnsCMV(no
intron)-FLgag-SPA - in order to compare them with the construct described above
possessing the intron A portion of the CMV promoter, pV1JnsHIVgag. Gag antibody
and Elispot responses (described in PCT International Application No.
PCT/US00/18332 (WO 01/02607) filed July 3, 2000, claiming priority to U.S.
Provisional Application Serial No. 60/142,631, filed July 6, 1999 and U.S.
Application Serial No. 60/148,981, filed August 13, 1999, all three applications which
are hereby incorporated by reference) were measured. The results displayed in Table
3 below, show that the new plasmid constructs behaved equivalently to the original
construct in Balb/c mice with respect to their antibody and T-cell responses at both
dosages of plasmid DNA tested, 20 μg and 200 μg.

⁵ b New plasmid constructions that have the intron A portion removed from the hCMV promoter.

^c In this construct the bGH terminator has been replaced with the short synthetic polyadenylation signal (SPA)

EXAMPLE 4

Table 3: HIV191: Immunogenicity of V1Jns-gag under different promoter and termination control elements.

DNA®	Dose, ug ^b		Anti-p24 Titers (3 Wk PD1) ^c		SFC/10^6 Cells (4 Wk PD1) ^d				
Promoter/terminator		GMT	+SE_	-SE	Media	gag197-205	p24		
HIVFL-gagPR9901	200	12800	4652	3412	2(2)	129(19)	30(11)		
(GMP grade)	20	5572	1574	1227	0	56(9)	25(6)		
pV1Jns-hCMV-	200	11143	2831	2257	0	98(5)	12(6)		
FL-gag-bGHpA	20	7352	2808	2032	0	73(9)	11(6)		
pV1Jns-hCMV-	200	16890	5815	4326	1(1)	94(4)	26(7)		
FL-gag-SPA	20	5971	5361	2825	0	85(17)	38(10)		
Naīve	0	123	50	36	0	0	0		

Ein PBS

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Construction of the Modified Shuttle Vector - "MRKpdelE1 Shuttle"

The modifications to the original Ad5 shuttle vector (pdelE1sp1A; a vector comprising Ad5 sequences from basepairs 1-341 and 3524-5798, with a multiple cloning region between nucleotides 341 and 3524 of Ad5, included the following three manipulations carried out in sequential cloning steps as follows:

- (1) The left ITR region was extended to include the *Pac1* site at the junction between the vector backbone and the adenovirus left ITR sequences. This allow for easier manipulations using the bacterial homologous recombination system.
- 10 (2) The packaging region was extended to include sequences of the wild-type (WT) adenovirus from 342 bp to 450 bp inclusive.
 - (3) The area downstream of pIX was extended 13 nucleotides (i.e., nucleotides 3511-3523 inclusive).
- These modifications (Figure 4) effectively reduced the size of the E1 deletion without overlapping with any part of the E1A/E1B gene present in the transformed PER.C6[®] cell line. All manipulations were performed by modifying the Ad shuttle vector pdelE1sp1A.

Once the modifications were made to the shuttle vector, the changes were incorporated into the original Ad5 adenovector backbones (pAdHVO and pAdHVE3) by bacterial homologous recombination using *E. coli* BJ5183 chemically competent cells.

bi.m. Injections into both quads, 50 μL per quad

cn=10;GMT, geometric mean titer; SE, standard, error

dn=5, pooled spleens; mean of triplicate wells and standard, deviation, in parentheses;

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EXAMPLE 5

Construction of Modified Adenovector Backbones (E3+ and E3-)

The original adenovectors pAdHVO (comprising all Ad5 sequences except those nucleotides encompassing the E1 and E3 regions) and pADHVE3 (comprising all Ad5 sequences except those nucleotides encompassing the E1 region), were each 5 reconstructed so that they contained the modifications to the E1 region. This was accomplished by digesting the newly modified shuttle vector (MRKpdelE1 shuttle) with Pac1 and BstZ1101 and isolating the 2,734 bp fragment which corresponds to the adenovirus sequence. This fragment was co-transformed with DNA from either Cla1 linearized pAdHVO (E3- adenovector) or Cla1 linearized pAdHVE3 10 (E3+adenovector) into E. coli BJ5183 competent cells. At least two colonies from each transformation were selected and grown in Terrific™ broth for 6-8 hours until turbidity was reached. DNA was extracted from each cell pellet and then transformed into E. coli XL1 competent cells. One colony from each transformation was selected and grown for plasmid DNA purification. The plasmid was analyzed by restriction 15 digestions to identify correct clones. The modified adenovectors were designated MRKpAdHVO (E3- plasmid) and MRKpAdHVE3 (E3+ plasmid). Virus from these new adenovectors (MRKHVO and MRKHVE3, respectively) as well as the old version of the adenovectors were generated in the PER.C6® cell lines to accommodate the following series of viral competition experiments. In addition, the multiple 20 cloning site of the original shuttle vector contained ClaI, BamHI, Xho I, EcoRV, HindIII, Sal I, and Bgl II sites. This MCS was replaced with a new MCS containing Not I, Cla I, EcoRV and Asc I sites. This new MCS has been transferred to the MRKpAdHVO and MRKpAdHVE3 pre-plasmids along with the modification made to the packaging region and pIX gene. 25

EXAMPLE 6

Analysis of the Effect of the Packaging Signal Extension

To study the effects of the modifications made to the E1 deletion region, the viruses obtained from the original backbone (pAdHVE3) and the new backbone (MRKpAdHVE3) were mixed together in equal MOI ratios (1:1 and 5:5) and passaged through several rounds; see Figure 5, Expt.#1. Both of the viruses in the experiment contained the E3 gene intact and did not contain a transgene. The only difference between the two viruses was within the region of the E1 deletion. Following the coinfection of the viruses at P1 (passage 1), the mixtures were propagated through an additional 4 passages at which time the cells were harvested

and the virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *Hind*III and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids (pAdHVE3 ("OLD E3+"); MRKpAdHVE3 ("NEW E3+")) were also digested with *Hind*III (and *Pac1* to remove the vector backbone) and subsequently labeled with [³³P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 clearly shows that the new adenovirus which has the addition made to the packaging signal region has a growth advantage compared with the original adenovirus. In the experiments performed (at either ratio tested), only the digestion bands pertaining to the newly modified virus were present. The diagnostic band of size 3,206 (from the new virus) was clearly present. However, there was no evidence of the diagnostic band of size 2,737 bp expected from the original virus.

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EXAMPLE 7

Analysis of the Effect of the E3 Gene

The second set of the virus competition study involved mixing equal MOI ratio (1:1) of the newly modified viruses, that obtained from MRKpAdHVO and MRKpAdHVE3 (Figure 5, Expt. #2). In this set, both viruses had the new modifications made to the E1 deletion. The first virus (that from MRKpAdHVO) does not contain an E3 gene. The second virus (that from MRKpAdHVE3) does contain the E3 gene. Neither of the viruses contain a transgene. Following coinfection of the viruses, the mixtures were propagated through an additional 4 passages at which time the cells were harvested and the total virus extracted and purified by CsCl banding. The viral DNA was extracted and digested with *HindIII* and the digestion products were then radioactively labeled. For the controls, the respective pre-plasmids MRKpAdHVO ("NEW E3-"); MRKpAdHVE3 ("NEW E3+") were also digested with HindIII (and Pac1 to remove the vector backbone) and then labeled with [33P]dATP. The radioactively labeled digestion products were subjected to gel electrophoresis and the gel was dried down onto Whatman paper before being exposed to autoradiographic film. Figure 6 shows the results of the viral DNA analysis of the E3+ virus and E3- virus mixing experiment. The diagnostic band corresponding to the E3+ virus (5,665 bp) was present in greater amount compared with the diagnostic band of 3,010 bp corresponding to the E3- virus. This indicates that the virus that contains the E3 gene is able to amplify more rapidly

compared with the virus that does not contain an E3 gene. This increased amplification capacity has been confirmed by growth studies; see Table 4 below.

EXAMPLE 8

Construction of the new shuttle vector containing modified gag transgene – "MRKpdelE1-CMV(no intron)-FLgag-bGHpA"

The modified plasmid pV1JnsCMV(no intron)-FLgag-bGHpA was digested with Msc1 overnight and then digested with Sfi1 for 2 hours at 50°C. The DNA was then treated with Mungbean nuclease for 30 mins at 30°C. The DNA mixture was desalted using the Qiaex II kit and then Klenow treated for 30 mins at 37°C to fully blunt the ends of the transgene fragment. The 2,559 bp transgene fragment was then gel purified. The modified shuttle vector (MRKpdelE1 shuttle) was linearized by digestion with EcoRV, treated with calf intestinal phosphatase and the resulting 6,479 bp fragment was then gel purified. The two purified fragments were then ligated together and several dozen clones were screened to check for insertion of the transgene within the shuttle vector. Diagnostic restriction digestion was performed to identify those clones carrying the transgene in the E1 parallel and E1 anti-parallel orientation. This strategy was followed to clone in the other gag transgenes in the MRKpdelE1 shuttle vector.

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EXAMPLE 9

Construction of the MRK FG Adenovectors

The shuttle vector containing the HIV-1 gag transgene in the E1 parallel orientation, MRKpdelE1-CMV(no intron)-FLgag-bGHpA, was digested with Pac1. The reaction mixture was digested with BsfZ171. The 5,291 bp fragment was purified by gel extraction. The MRKpAdHVE3 plasmid was digested with Cla1 overnight at 37°C and gel purified. About 100 ng of the 5,290 bp shuttle +transgene fragment and -100 ng of linearized MRKpAdHVE3 DNA were co-transformed into E. coli BJ5183 chemically competent cells. Several clones were selected and grown in 2 ml TerrificTM broth for 6-8 hours, until turbidity was reached. The total DNA from the cell pellet was purified using Qiagen alkaline lysis and phenol chloroform method. The DNA was precipitated with isopropanol and resuspended in 20 μl dH₂0. A 2 μl aliquot of this DNA was transformed into E. coli XL-1 competent cells. A single colony from each separate transformation was selected and grown overnight in 3 ml LB +100 μg/ml ampicillin. The DNA was isolated using Qiagen columns. A positive clone was identified by digestion with the restriction enzyme BstEII which cleaves

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within the gag gene as well as the plasmid backbone. The pre-plasmid clone is designated MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA and is 37,498 bp in size. This strategy was followed to generate E3- and E3+ versions of each of the other gag transgene constructions in both E1 parallel and E1 anti-parallel versions. Figures 7A, 7B and 7C show the various combinations of adenovectors constructed.

EXAMPLE 10

Plasmid Competition Studies

A series of plasmid competition studies was carried out. Briefly, the screening of the various combinations of new constructs was performed by mixing equal amounts of each of two competing plasmids. In the experiment shown in Figure 8A, plasmids containing the same transgene but in different orientations were mixed together to create a "competition" between the two plasmids. The aim was to look at the effects of transgene orientation. In the experiment shown in Figure 8B, plasmids containing different polyadenylation signals (but in the same orientation) were mixed together in equal amounts. The aim was to assess effects of polyA signals. Following the initial transfection, the virus was passaged through ten rounds and the viral DNA analyzed by radioactive restriction analysis.

Analysis of the viral species from the plasmid mixing experiment (Figure 8A) showed that adenovectors which had the transgene inserted in the E1 parallel orientation amplified better and were able to out-compete the adenovirus which had the transgene inserted in the E1 anti-parallel orientation. Viral DNA analysis of the mixtures at passage 3 and certainly at passage 6, showed a greater ratio of the virus carrying the transgene in the E1 parallel orientation compared with the E1 antiparallel version. By passage 10, the only viral species observed was the adenovector with the transgene in the E1 parallel orientation for both transgenes tested (hCMV(no intron)-FLgag-bGHpA and hCMV(no intron)-FLgag-SPA).

Analysis of the viral species from the plasmid mixing experiment #2 (Figure 8B) at passages 3 and 6 showed that the polyadenylation signals tested (bGHpA and SPA) did not have an effect on the growth of the virus. Even at passage 10 the two viral species in the mixture were still present in equal amounts.

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EXAMPLE 11

Virus generation of an enhanced adenoviral construct - "MRK Ad5 HIV-1gag"

The results obtained from the competition study allowed us to make the following conclusions: (1) The packaging signal extension is beneficial; (2) Presence of E3 does enhance viral growth; (3) E1 parallel orientation is recommended; and (4) PolyA signals have no effect on the growth of the adenovirus.

MRK Ad5 HIV-1 gag exhibited the most desirable results. This construct contains the hCMV(no intron)-FLgag-bGHpA transgene inserted into the new E3+ adenovector backbone, MRKpAdHVE3, in the E1 parallel orientation. We have designated this adenovector MRK Ad5 HIV-1 gag. This construct was prepared as outlined below:

The pre-plasmid MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA was digested was Pac1 to release the vector backbone and 3.3 µg was transfected by calcium phosphate method (Amersham Pharmacia Biotech.) in a 6 cm dish containing PER.C6[®] cells at ~60% confluence. Once CPE was reached (7-10 days), the culture was freeze/thawed three times and the cell debris pelleted. 1 ml of this cell lysate was used to infect into a 6 cm dish containing PER.C6® cells at 80-90% confluence. Once CPE was reached, the culture was freeze/thawed three times and the cell debris pelleted. The cell lysate was then used to infect a 15 cm dish containing PER.C6[®] cells at 80-90% confluence. This infection procedure was continued and expanded at passage 6. The virus was then extracted from the cell pellet by CsCl method. Two bandings were performed (3-gradient CsCl followed by a continuous CsCl gradient). Following the second banding, the virus was dialyzed in A105 buffer. Viral DNA was extracted using pronase treatment followed by phenol chloroform. The viral DNA was then digested with *Hind*III and radioactively labeled with [33P]dATP. Following gel electrophoresis to separate the digestion products the gel was dried down on Whatman paper and then subjected to autoradiography. The digestion products were compared with the digestion products from the pre-plasmid (that had been digested with Pacl/HindIII prior to labeling). The expected sizes were observed, indicating that the virus had been successfully rescued. This strategy was used to rescue virus from each of the various adenovector plasmid constructs prepared.

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EXAMPLE 12

Stability Analyses

To determine whether the various adenovector constructs (e.g., MRK Ad5 HIV-1 gag) show genetic stability, the viruses were each passaged continually. The viral DNA was analyzed at passages 3, 6 and 10. Each virus maintained its correct genetic structure. In addition, the stability of the MRK Ad5 HIV-1 gag was analyzed under propagation conditions similar to that performed in large scale production. For this analysis, the transfections of MRK Ad5 HIV-1 gag as well as three other adenoviral vectors were repeated and the virus was purified at P3. The three other adenovectors were as follows: (1) that comprising hCMV(no intron)-Flgag with a bGHpA terminator in an E3- adenovector backbone; (2) that comprising hCMV(no intron)-Flgag with a SPA termination signal in an E3+ adenovector backbone, and that comprising a mCMV-Flgag with a bGHpA terminator in an E3+ adenovector backbone. All of the vectors have the transgene inserted in the E1 parallel orientation. Viral DNA was analyzed by radioactive restriction analysis to confirm that it was correct before being delivered to fermentation cell culture for continued passaging in serum-free media. At P5 each of the four viruses were purified and the viral DNA extracted for analysis by the restriction digestion and radiolabeling procedure. This virus has subsequently been used in a series of studies (in vitro gag expression in COS cells, rodent study and rhesus monkey study) as will be described below. The viruses from P5 are shown in Figure 9.

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The passaging under serum-free conditions was continued for the MRKHVE3 (transgene-less, obtained from MRKpAdHVE3 pre-plasmid) and the MRKAd5HIV-1gag (obtained from MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA pre-plasmid) viruses. Figure 10 shows viral DNA analysis by radioactive restriction digestion at passage 11 for MRKHVE3, MRKAd5HIV-1gagE3-, and passage 11 and 12 for MRKAd5HIV-1gag. Aside from the first lane which is the DNA marker lane, the next three lanes are virus from the pre-plasmid controls (controls based on the original virus) - MRKpAdHVE3 (also referred to as "pMRKHVE3"), MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA, and pMRKAd5gag(E3-), respectively. As seen in Figure 10, each of the viral DNA samples show the expected bands with no extraneous bands showing. This signifies that there are no major variant adenovirus species present that can be detected by autoradiography.

Figure 11 shows the results of viral competition study between MRKHVE3 and MRKAd5HIV-1gag. These viruses were mixed together at equal MOI (140 viral

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particles each; 280 vp total) at passage 6 and continued to be passaged until P11. Aside from the first lane which is the DNA marker lane, the next two lanes are the pre-plasmid controls obtained from MRKpAdHVE3 and MRKpAdHVE3+CMV(no intron)-FLgag-bGHpA. The next two lanes are the viral DNA from the starting viral material at passage six. The last two lanes are the competition studies performed in duplicate. The data in Figure 11 shows the effect the gag transgene in culture. Growth of a MRKAd5gag virus was compared with growth of a "transgene-less" MRKHVE3. These two viruses were infected at the same MOI (i.e. 140 vp each) at passage 6 and then passaged through to passage 11 and the viral pool was analyzed by radioactive restriction analysis. The data shows that one virus did not out compete the other. Therefore, the gag transgene did not show obvious signs of toxicity to the adenovirus.

Analysis by *Hind*III digestion shows that each virus specie is present in approximately equal amounts. As above, there does not appear to be signs of any extraneous bands. Figure 12 shows higher passage numbers for MRKAd5HIV-1gag grown under serum-containing conditions. The genome integrity again has been maintained and there is no evidence of rearrangements, even at the highest passage level (P21).

Each of the four vectors shown in Figure 9 were analyzed for amplification capacity. Table 4 below shows the QPA analysis used in the estimation of viral amplification ratios at P4. The determination of the amplification ratio for the original HIV-1 gag construct is based on the clinical lot at P12. It has been shown that amplification rates increases with higher passage number for the original virus. The reason for this observation is due to the emergence of variants which exhibit increased growth rates compared to the intact adenovector. With continued passaging of the original Ad gag vector, the level of variants increases and hence amplification rates increase also.

The MRK Ad5 HTV-1 gag virus has also been continually passaged under process conditions (i.e., serum-free media). Viral DNA extracted from passages 11 and 12 show no evidence of rearrangement.

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Table 4:
Amplification Ratios Based on AEX and QPA Analysis of Virus Amplification from Passage 3 to Passage 4.

Ad gag construct	Amplification Ratio
MRKAd5gag	470
HCMV-Flgag-bGHpA [E3-]	115
HCMV-Flgag-SPA [E3+]	320
mCMV-FLgag-bGHpA [E3+]	420
Original construct *	40 - 50

^{*} This estimation is based on the clinical lot growth characteristics at Passage 12.

EXAMPLE 13

Analytical Evaluation of the enhanced Ad5 Constructs

To study the effects of the transgene and the E3 gene on virus amplification, the enhanced adenoviral vector, MRK Ad5 HIV-1 gag, along with its transgene-less version (MRKpAdHVE3) and its E3- version (MRK Ad5 HIV-1 gag E3-), was studied for several passages under serum-free conditions. Table 5A shows the amplification ratios determined for passages P3 to P8 for MRK Ad5 HIV-1 gag. Within a certain MOI range, it has been determined that the virus output is directly proportional to the virus input. Therefore, the greater the number of virus particles per cell at infection, the greater the virus amount produced. Viral amplification ratios, on the other hand, are inversely proportional to the virus input. The lower the virus input, the greater the amplification ratio.

Table 5B shows the amplification rates of the new E3+ vector backbone MRKpAdHVE3. It has a significantly lower rate of amplification compared with the gag transgene containing version. This may be contributed to the larger size MRK Ad5 HIV-1 gag since it contains the transgene. This inclusion of the transgene brings the size of the adenovirus closer to the size of a wild type Ad5 virus. It is well known that adenoviruses amplify best when they are at close to their wild type genomic size.

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Wild type Ad5 is 35,935 bp. The MRKpAdHVE3 is 32,905 bp in length. The enhanced adenovector MRK Ad5 HIV-1 gag is 35,453bp (See Figure 14 for vector map; see also Figure 15A-X show the complete pre-adenoviral vector sequence, which includes an additional 2,021 bp of the vector backbone).

Table 5C shows the amplification rates of the new E3- gag containing virus MRK Ad5 HIV-1 gag E3-. Once again, this virus shows lower growth rate than the enhanced adenoviral vector. This may be attributed to the decreased sized of this virus (due to the E3 gene deletion) compared with wild type Ad5. The MRK Ad5 HIV-1 gag E3- virus is 32,810 bp in length. This can be compared with the wild type Ad5 which is 35,935 bp and MRK Ad5 HIV-1 gag which is 35,453 bp in length.

Table 5A: Amplification ratios determined by AEX and QPA for MRKAd5gag over several continuous passaging in serum free media. Following P5, two replicate samples were taken (rep-1 and rep-2) and analyzed.

MRKAd5gag rep1

	XV (10° cells/ii	I), Viability (%)	Harvest Time	Cell Passage	Titer	Ther	QPA	Ratio	Amplification	AEX
	Infection	Harvest	hpl	Number	10 ¹⁰ vp/ml culture	10° vp/cell	10° TCIDe/mi	AEXCOPA	Ratio	Internal Control
P4	1,49, 81%	0.58, 50%	44	46	8.7	5.9	1.72	50	470 (MOI = 125)	
P5	1.38, 93%	0.66, 47%	48	49	6.7	4.9	1.38	49	170	
P6	1.04, 94%	0.68, 77%	47	48	5.8	5.6	1.42	41	200	
P7	1,50, 84%	0.88, G1%	49.5	50	3.9	1.4	0.97	40	50	
P7	1.09, 97%	0.78, 59%	50	52	5.2	4.7	1.70	81	170	
P8	1.03, 94%	0.88, 64%	47.5	54	9.0	8.7	1.10	62	310	0.00
P9	0.89, 95%	0.99, 73%	47.5	56	4.4	4.9	1.03	43	175	3.12 2.84
P10	1,09, 91%	1.08, 65%	47.5	58	8.0	2.8	1.16	26	100	2.70 2.60
P11	1.19, 88%	0.98, 65%	47	60	3.6	3.0	1.15	31	110	2.70 2.70
P12	0.98, 91%	0.85, 63%	47.5	47	5.4	5.5	1.20	45	200	2.68 2.60
P13	1.00, 88%	0.70, 87%	49	49	5.8	5.8	1.11	52	210	3.18 3.18
P14	1,94, 92%	0.88, 67%	46	53	8.6	4.4			160	3.28 3.27
P15	0.97, 96%	0.64, 66%	47	47	6.9	7.1			250	3.12 2.91

Table 5B: Amplification ratios determined by AEX and QPA for MRKHVE3 over several continuous passaging in serum free media. MRKHVE3 is the new vector backbone which does NOT carry a transgene.

MRKHVE3

	Xv (10° cells/r Infection	ni), Viability (%) Harvest	Harvest Time h.p.t.	Cell Passage Number	Ther 10 ¹⁰ vp/ml culture	Titer 10° vp/cell	QPA 10° TCID _{so} /ml	Ratio AEX:QPA	Amplification Ratio	AEX Internal Control
P4	1.10, 97%	1.28, 79%	49	54	4.1	3.8	1.70	25	300 (MOI = 125)	
P5	0.82, 89%	1.18, 77%	47	. 48	4.3	4.7	1.24	35	170	
P6	1,55, 88%	1.26, 76%	49.5	60	1.2	0.8	0.58	21	30	
P6	1.09, 97%	1.11,81%	49	52	4.0	3.6	1.16	34	130	
P7	1.17, 91%	1.22, 91%	47.5	54	3.7	3.2	0.50	74	110	Ì
P8	0.98, 88%	1,41, 83%	48	56	2,1	2.1	0.47	45	75	3.12 2.84
Pg	1.20, 89%	1.26, 81%	47.5	58	0.8	0.7	0.29	28	25	2.70 2.60
P10	0.99, 82%	1.65, 88%	47	60	2.3	2.3	0.43	53	80	2.70 2.70
P11	1.07, 96%	1.25, 83%	48	47	2.7	2.5	0.41	66	90	2.86 2.60
P12	0.80, 91%	1.14, 80%	49.5	49	5.9	7.4	0.48	123	280	3.18 3.18
P13	1.98, 95%	1.14, 65%	45.5	53	5.8	3.0			110	3.28 3.27
P14	0.97, 96%	1.03, 98%	48.5	47	9.4	9.7			350	3.12 2.91
P15	0.87, 99%	0.97, 59%	49.5	49	5.3	6.1		1	218	2.78 2.52

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Table 5C. Amplification ratios determined by AEX and QPA for MRKAd5gag(E3-) over several continuous passaging in serum free media. This construct is identical to the MRKAd5gag construct except that this version is DELETED of the E3 gene.

MRKAd5gag(E3-)

	Xv (10° cells/ml), Vlability (%)		Harvest Time	Cell Passage	Titer	Titer	QPA .	Flatio	Amplification	AEX
	Infection	Harvest	h.p.l.	Number	10 to vp/ml culture	10° vp/cell	10° TCID _{EP} /ml	AEX:QPA	Ratio	Internal Control
P4	1.62, 77%	1.12, 62%	47.5	46	2.0	1.2	0.92	20	100 (MOI=125)	
P5	1.16, 92%	0.62, 43%	49	49	3.3	2.9	0.99	34	100	
P6	1.71, 86%	0.20, 10%	49	50	4.7	2.7	1.70	28	100	
P6	1.09, 97%	0.63, 54%	49.5	52	5.4	5.0	1.76	31	180	
P7	1.17, 91%	0.98, 72%	47.50	54	7.1	6.1	0.67	106	220	
P8	0.98, 88%	0.77, 48%	48	56	3.1	3.2	0.68	47	115	3.12 2.84
P9	1.20, 89%	1.03, 72%	48	58	1.8	1.5	0.57	32	65	2.70 2.60
P10	0.99, 82%	0.80, 62%	46.5	60	3.2	3.2	0.68	47	115	2.70 2.70
P11	1.07, 96%	0.98, 70%	48.5	47	5.9	5.5	0.68	87	200	2.88 2.60
P12	0.80, 91%	0.67, 59%	50	49	5.1	6.4	0.72	71	230	3.18 3.18
P13	1.96, 95%	0.91, 59%	45.5	53	7.4	3.8			135	3.28 3.27
P14	0.97, 96%	0.81, 74%	48	47	6.8	7.0	<u> </u>		250	3.12 2.81
P15	0.87, 99%	0.84, 56%	49	49	4,8	5.5			196	2.78 2.52

EXAMPLE 14

Gag Expression Analysis of the Novel Constructs

In vitro gag analysis of the MRK Ad5 HIV-1 gag and the original HIV-gag vectors (research and clinical lot) show comparable gag expression. The clinical lot shows only a slightly reduced gag expression level. The most noticeable difference is with the mCMV vector. This vector shows roughly 3 fold lower expression levels compared with the other vectors tested (which all contain hCMV promoters). The mCMV-FLgag with bGHpA assay was performed three times using different propagation and purification lots and it consistently exhibited weaker gag expression.

EXAMPLE 15

Evaluation of MRK Ad5 HIV-1 gag and Other gag-Containing Adenovectors in Balb/c Mice

Cohorts of 10 balb/c mice were vaccinated intramuscularly with escalating doses of MRK Ad5 HIV-1 gag, and the research and clinical lots of original Ad5HIV-1gag. Serum samples were collected 3 weeks post dose 1 and analyzed by anti-p24 sandwich ELISA.

Anti-p24 titers in mice that received MRK Ad5 HIV-1 gag (107 and 109 vp(viral particle) doses) were comparable (Figure 13) to those of the research lot of Ad5HIV-1 gag, for which much of the early rhesus data were generated on. These titers were also comparable when E3 is deleted (MRKAd5hCMVgagbGHpA(E3-)) or SPA is substituted for bGHpA terminator (MRKAd5 hCMV-gag-SPA (E3+)) or murine CMV promoter is used in place of hCMV (MRKAd5 mCMV-gag-bGHpA (E3+)) in the MRKAd5 backbone.

The results shown in Table 7 indicate that the three other vectors (in addition to the preferred vector, MRK Ad5 HIV-1 gag, are also capable of inducing strong anti-gag antibody responses in mice. Interestingly enough, while the mCMV-FLgag construct containing bGHpA and E3+ in an E1 parallel orientation showed lowest gag expression in the COS cell *in vitro* infection (Table 6) in comparison with the other vectors tested, it generated the greatest anti-gag antibody response this *in vivo* Balb/c study. Table 7 also shows a dose response in anti-gag antibody production in both the research and the clinical lot. As expected, the clinical lot shows reduced anti-gag antibody induction at each dosage level compared to the same dosage used for the research lot.

Table 6: In vitro analysis for gag expression in COS cells by Elisa assay.

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Viral Vectors ^a	μg gag/4.8x10e5 COS/10e8 parts/48hr
MRKAd5gag ^b	1.40
Clinical lot Ad5gag ^c	1.28
Research lot Ad5gag ^d	1.32
MCMVFL-gagbGHpA ^c	0.42

^a A_{260nm} absorbance readings taken for viral particle determinations.

^b MRKAd5gag was produced in serum free conditions and purified at P5.

^c Clinical lot# Ad5gagFN0001

²⁵ dResearch Ad5FLgag lot# 6399

^e mCMVFL-gagbGHpA was produced in serum free conditions and purified at P5.



Table 7: mHIV020 Anti-p24 Ab Titers in Balb/c mice (n=10) vaccinated with various Adgag constructs and lots (3 week post dose1).

Group ID	Vaccine	Dose (vp)	GMT	SE upper	SE lower
1	^a MRKAd5gag	10^7	25600	5877	4780
2	в	10^9	409600	94028	76473
3	hCMV FL-gag bGHpA [E3-] →	10^7	7352	2077	1620
4		10^9	235253	59767	47659
5	hCMV FL-gag SPA [E3+] →	10^7	12800	9905	236
6	, , ,	10^9	310419	99181	75165
7	bmCMV FL-gag bGHpA [E3+] →	10^7	44572	23504	15389
8	•	10^9	941014	239068	190636
9	^c hCMV FL-gag bGHpA [E3-] ←	10^7	3676	934	745
10		10^9	117627	17491	15227
11	research lot hCMV intronA FL-gag bGHpA [E3-] <-	10^6	528	262	175
12		10^7	14703	5274	3882
13		10^8	58813	14942	11915
14	Ţ	10^9	204800	53232	42250
15	clinical lot hCMVintronA FL-gag bGHpA [E3-] <-	10^6	230	82	61
16		10^7	4222	3405	1138
17	•	10^8	19401	3939	3274
18		10^9	89144	25187	19639
19	Naïve	none	93_	7	6

*2x50 µL i.m. (quad) injections/animal

P.I.s: Youil, Chen, Casimiro Vaccination: T. Toner, Q. Su

Assay: M. Chen

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^aThe structure of MRKAd5gag is: hCMVFL-gagbGHpA [E3+] → The <u>same lot</u> of MRKAd5gag used in this rodent study was used in the Rhesus monkey study (Tables 7 and 8).

^bThe same lot of mCMVFL-gagbGHpA[E3+] used in the *in vitro* study (Table 6) ws used here.

^cThis construct was designed by Volker Sandig. It contains a shorter version of the hCMV promoter than that used in the MRK constructs. The adenovector backbone is identical to the original backbone used in the original Adgag vector. Expression at 10e7 dose from this vector is 7 fold lower then the same dose of the MRKAd5gag and 4 fold lower than the research lot.

EXAMPLE 16

Comparison of Humoral and Cellular Responses Towards the Original Ad-gag Construct with the New MRK Ad5 HIV-1 gag in Rhesus Monkeys

Cohorts of 3 rhesus monkeys were vaccinated intramuscularly with MRK Ad5 HIV-1 gag or the clinical Ad5gag bulk at two doses, 10^{11} vp and 10^{9} vp.

Immunizations were conducted at week 0, 4, and 25. Serum and PBMC samples were collected at selected time points. The serum sample were assayed for anti-p24 Ab titers (using competitive based assay) and the PBMCs for antigen-specific IFN-gamma secretion following overnight stimulation with gag 20-mer peptide pool (via ELISpot assay).

The results shown in Table 8 indicate comparable responses with respect to the generation of anti-gag antibodies. The frequencies of gag-specific T cells in

peripheral blood assummarized in Table 9 demonstrate a strong cellular immune response generated after a single dose with the new construct MRK Ad5 HIV-1 gag. The responses are also boostable with second dose of the same vector. The vector is also able to induce CD8+ T cell responses (as evident by remaining spot counts after CD4+ depletion of PBMCs) which are responsible for cytotoxic activity.

Table 8 Anti-p24 antibody titers (in mMU/mL) in rhesus macaques immunized with

gag-expressing adenovectors (Protocol HIV203).

Vaccine	Pre	Wk4	Wk8	Wk 12	Wk 16	Wk 20	Wk 25	Wk 28
	P P I B	WK4	AAKO	WKIZ	VVK 10	VV ZU	W K 23	V/ K 20
MRKAd5gag ^a , 10^11 vp								
97N010	<10	118	5528_	11523	7062	21997	ND	51593
97N116	<10	62	772	1447	1562_	2174	ND	20029
98X007	<10	66∙	3353	6156	6845	3719	ND	24031
MRKAd5gog, 10^9 vp								
97N120	<10	_51	204	318	366	482	ND	6550
97N144	<10	18	118	274	706	888	ND	7136
98X008	<10	15	444	386	996	1072	ND	12851
Ad5gagb, Clinical Lat, 10^11 vp								
97X001	<10	87	2579_	4718	7174	7250	ND	69226
97N146	<10	72	3604	7380	7526	18906	ND_	60283
98X009	<10	78	4183	3946	3124	6956	ND	26226
Ad5gag, Clinical Lat, 10^9 vp								
97N020	<10	<10	143	371	390	1821	ND	17177
97X003	<10	<10	39	93	156	596	ND	2053
98X012	<10	_81	342	717	956	1558	ND	11861
MRKAd5gag (hCMV, bGHpA, E3+)								
boriginal Actigag vector (hCMV/Intro	n A bGHp	A, E3-), lott	FN0001_			<u> </u>		
ND, not determined				<u> </u>	<u></u>	<u> </u>	<u> </u>	

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Table 9. Number of gag-specific T cells per million peripheral blood mononuclear cells (PBMCs) in rhesus monkeys immunized with gag-expressing adenovectors. Also included are those frequencies in PBMCs depleted of CD4⁺ T cells.

Vaccination	Monkey ID			T=4 Wk					Wk						Wk
T=0,4,25 wks		Media	Gog H	Media	Gog H	Medio	Gog H	Mediq	GogH	Media	Gog H	Media	Goog H		
MRKACEGOO	97N010	6	89	o	395	0	1058	0	1174	3	775 76	4	1074 594		
10~11 Vp		1 1	398	1	609	ő	534	4	395	lĭ	261	lŏ	408		
	97N116(CD4-)	11	676	1		ō	593			0	184	0	666		
	98X007	10	579	0	1304	3	2193	וו	2118				2113		
	98X007(CD4-)	20	965			0	2675			0	1656	0	1278		
MRKAc5gcg	97N120	5	275	1	249	4	141	4	119	9	206	4	219		
10'9 Vp								١.	051			!!	219 373		
				1 6	438			3	250				625		
		_		١.	1000			١,	A73				735		
	98X008(CD4-)	14	696	'	1.000	ő	1175	-	5,5	ŏ	391	1 4	848		
Adface dinical lat	97X001	0	261	1	485	0	817	0	12200	1	894	0	1858		
10^11 vp	97X001(CD4-)	10											1123		
		-		1	465			1	1272				1785		
				١.				1	- m				971		
				1 3	339			١ ٠	840				644		
	98X009(CD4-)	0	/3			<u> </u>	333			L °		L			
AdSaca dinical lat	97N020	3	30	1	101	0	66	0	36	0	26	0.0	41 16		
tu-a Ab				В	134			1 1	38		38		aī aī		
		0	40	l	1	Ιŏ	١٠٠	1	"	Ö	4	Ιŏ	19		
	98X012	5	95	3	54	l i	34	0	18	0	20	i	121		
	98X012(CD4-)	11	70			0	11			0	8	0	41		
Nave	96R041	6	8	1	1	20	0	0	0	0,	0	1 20	0		
	MRKAGGCQ 10/91 vp MRKAGGCQ 10/9 vp MRKAGGCQ 10/9 vp	T=0.4.25 w/s MRKAc5ccccccccccccccccccccccccccccccccccc	T=0,4,25 w/s Medic*	T=0.4.25 w/s Media* Goa H*	T=0.4.25 yr/s	T=0,4,25 w/ss	T=0,4,25 yks	T=0,4,25 w/s Media Gog H Media Gog H	T=0,4,25 wks	T=0.4.25 yrks	T=0.4.25 y/ks	T=0,4,25 yks	T=0,4,25 w/s Medic Gog H		

Based on either 4x10/6 or 2x10/6 cells per well (depending on spot density)

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The adenovectors described herein and, particularly, MRK Ad5 HIV-1 gag, represent very promising HIV-gag adenovectors with respect to their enhanced growth characteristics in both serum and, more importantly, in serum-free media conditions. In comparison with the current HIV-1 gag adenovector construct, MRK Ad5 HIV-1 gag shows a 5-10 fold increased amplification rate. We have shown that it is genetically stable at passage 21. This construct is able to generate significant cellular immune responses in vivo even at a relatively low dose of 10^9 vp. The potency of the MRKAd5gag construct is comparable to, if not better than the original HIV-1gag vector as shown in this rhesus monkey study.

EXAMPLE 17 CODON OPTIMIZED HIV-1 POL AND CODON OPTIMIZED HIV-1 POL MODIFICATIONS

The open reading frames for the various synthetic *pol* genes disclosed herein comprise coding sequences for the reverse transcriptase (or RT which consists of a polymerase and RNase H activity) and integrase (IN). The protein sequence is based

N.D. not deterrithed

[&]quot;mock or no peptide control

Pool of 20-co peptides overlapping by 10 as and encompassing the gas sequence

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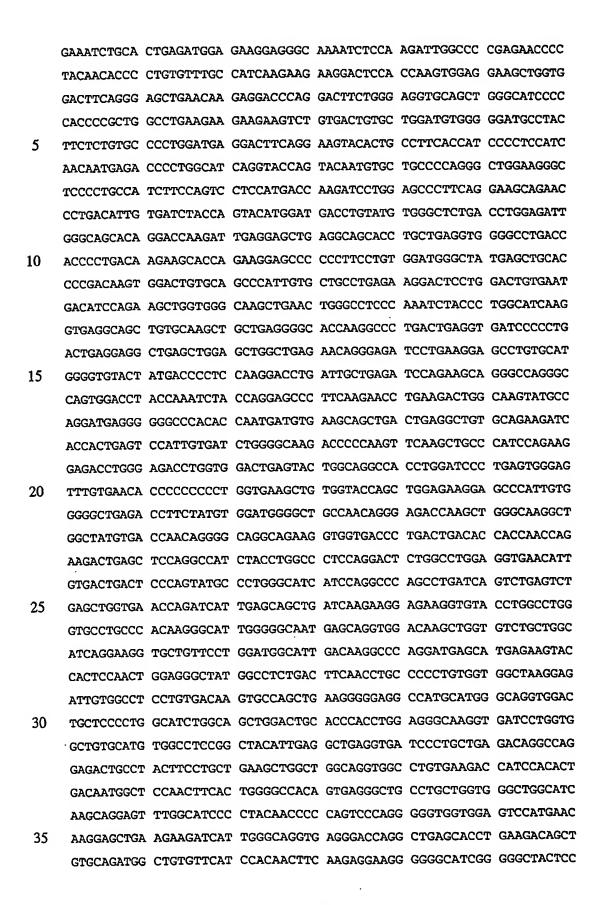
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on that of Hxb2r, a clonal isolate of IIIB; this sequence has been shown to be closest to the consensus clade B sequence with only 16 nonidentical residues out of 848 (Korber, et al., 1998, Human retroviruses and AIDS, Los Alamos National Laboratory, Los Alamos, New Mexico). The skilled artisan will understand after review of this specification that any available HIV-1 or HIV-2 strain provides a potential template for the generation of HIV pol DNA vaccine constructs disclosed herein. It is further noted that the protease gene is excluded from the DNA vaccine constructs of the present invention to insure safety from any residual protease activity in spite of mutational inactivation. The design of the gene sequences for both wildtype (wt-pol) and inactivated pol (IA-pol) incorporates the use of human preferred ("humanized") codons for each amino acid residue in the sequence in order to maximize in vivo mammalian expression (Lathe, 1985, J. Mol. Biol. 183:1-12). As can be discerned by inspecting the codon usage in SEQ ID NOs: 1, 3, 5 and 7, the following codon usage for mammalian optimization is preferred: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which, as noted elsewhere in this specification, is hereby incorporated by reference. It is intended that the skilled artisan may use alternative versions of codon optimization or may omit this step when generating HIV pol vaccine constructs within the scope of the present invention. Therefore, the present invention also relates to non-codon optimized versions of DNA molecules and associated recombinant adenoviral HIV vaccines which encode the various wild type and modified forms of the HIV Pol protein disclosed herein. However, codon optimization of these constructs is a preferred embodiment of this invention.

A particular embodiment of this portion of the invention comprisies codon optimized nucleotide sequences which encode wt-pol DNA constructs (herein, "wt-pol" or "wt-pol (codon optimized))" wherein DNA sequences encoding the protease (PR) activity are deleted, leaving codon optimized "wild type" sequences which encode RT (reverse transcriptase and RNase H activity) and IN integrase activity. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:1, the open reading frame being contained from an initiating Met residue at nucleotides 10-12 to a termination codon from nucleotides 2560-2562. SEQ ID NO:1 is as follows:

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC

ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG



GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGCC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ
ID NO:1).

The open reading frame of the wild type pol construct disclosed as SEQ ID NO:1 contains 850 amino acids, disclosed herein as SEQ ID NO:2, as follows: Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro 10 Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Asp 15 Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly 20 Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile 25 Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys 30 Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu 35 Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala

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Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro 10 Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val 15 Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly 20 Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp 25 Glu Asp (SEQ ID NO:2).

The present invention especially relates to an adenoviral vector vaccine which comprises a codon optimized HIV-1 DNA pol construct wherein, in addition to deletion of the portion of the wild type sequence encoding the protease activity, a combination of active site residue mutations are introduced which are deleterious to HIV-1 pol (RT-RH-IN) activity of the expressed protein. Therefore, the present invention preferably relates to an adenoviral HIV-1 DNA pol-based vaccine wherein the construct is devoid of DNA sequences encoding any PR activity, as well as containing a mutation(s) which at least partially, and preferably substantially, abolishes RT, RNase and/or IN activity. One type of HIV-1 pol mutant which is part and parcel of an adenoviral vector vaccine may include but is not limited to a mutated

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DNA molecule comprising at least one nucleotide substitution which results in a point mutation which effectively alters an active site within the RT, RNase and/or IN regions of the expressed protein, resulting in at least substantially decreased enzymatic activity for the RT, RNase H and/or IN functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct in a DNA molecule which contains at least one point mutation which alters the active site of the RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished. Such a HIV-1 Pol mutant will most likely comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. To this end, an especially preferred HIV-1 DNA pol construct is exemplified herein and contains nine codon substitution mutations which results in an inactivated Pol protein (IA Pol: SEQ ID NO:4, Figure 17A-C) which has no PR, RT, RNase or IN activity, wherein three such point mutations reside within each of the RT, RNase and IN catalytic domains. Therefore, an especially preferred exemplification is an adenoviral vaccine which comprises, in an appropriate fashion, a DNA molecule which encodes IA-pol, which contains all nine mutations as shown below in Table 1. An additional preferred amino acid residue for substitution is Asp551, localized within the RNase domain of Pol. Any combination of the mutations disclosed herein may suitable and therefore may be utilized as an IA-Pol-based vaccine of the present invention. While addition and deletion mutations are contemplated and within the scope of the invention, the preferred mutation is a point mutation resulting in a substitution of the wild type amino acid with an alternative amino acid residue.

\mathbf{T}	ab.	le	1

	wt aa	aa residue	mutant aa	enzyme function
	Asp	112	Ala	RT
	Asp	187	Ala	RT
30	Asp	188	Ala	RT
	Asp .	445	. Ala	. RNase H
	Glu	480	Ala	RNase H
	Asp	500	Ala	RNase H
	Asp	626	Ala	IN
35	Asp	678	Ala	IN
	Glu	714	Ala	IN

It is preferred that point mutations be incorporated into the IApol mutant adenoviral vaccines of the present invention so as to lessen the possibility of altering epitopes in and around the active site(s) of HIV-1 Pol.

To this end, SEQ ID NO:3 discloses the nucleotide sequence which codes for a codon optimized pol in addition to the nine mutations shown in Table 1, disclosed as follows, and referred to herein as "IApol":

AGATCTACCA TGGCCCCCAT CTCCCCCATT GAGACTGTGC CTGTGAAGCT GAAGCCTGGC ATGGATGGCC CCAAGGTGAA GCAGTGGCCC CTGACTGAGG AGAAGATCAA GGCCCTGGTG GAAATCTGCA CTGAGATGGA GAAGGAGGGC AAAATCTCCA AGATTGGCCC CGAGAACCCC 10 TACAACACCC CTGTGTTTGC CATCAAGAAG AAGGACTCCA CCAAGTGGAG GAAGCTGGTG GACTTCAGGG AGCTGAACAA GAGGACCCAG GACTTCTGGG AGGTGCAGCT GGGCATCCCC CACCCCGCTG GCCTGAAGAA GAAGAAGTCT GTGACTGTGC TGGCTGTGGG GGATGCCTAC TTCTCTGTGC CCCTGGATGA GGACTTCAGG AAGTACACTG CCTTCACCAT CCCCTCCATC AACAATGAGA CCCCTGGCAT CAGGTACCAG TACAATGTGC TGCCCCAGGG CTGGAAGGGC TCCCCTGCCA TCTTCCAGTC CTCCATGACC AAGATCCTGG AGCCCTTCAG GAAGCAGAAC 15 CCTGACATTG TGATCTACCA GTACATGGCT GCCCTGTATG TGGGCTCTGA CCTGGAGATT GGGCAGCACA GGACCAAGAT TGAGGAGCTG AGGCAGCACC TGCTGAGGTG GGGCCTGACC ACCCCTGACA AGAAGCACCA GAAGGAGCCC CCCTTCCTGT GGATGGGCTA TGAGCTGCAC CCCGACAAGT GGACTGTGCA GCCCATTGTG CTGCCTGAGA AGGACTCCTG GACTGTGAAT 20 GACATCCAGA AGCTGGTGGG CAAGCTGAAC TGGGCCTCCC AAATCTACCC TGGCATCAAG GTGAGGCAGC TGTGCAAGCT GCTGAGGGGC ACCAAGGCCC TGACTGAGGT GATCCCCCTG ACTGAGGAGG CTGAGCTGGA GCTGGCTGAG AACAGGGAGA TCCTGAAGGA GCCTGTGCAT GGGGTGTACT ATGACCCCTC CAAGGACCTG ATTGCTGAGA TCCAGAAGCA GGGCCAGGGC CAGTGGACCT ACCAAATCTA CCAGGAGCCC TTCAAGAACC TGAAGACTGG CAAGTATGCC 25 AGGATGAGGG GGGCCCACAC CAATGATGTG AAGCAGCTGA CTGAGGCTGT GCAGAAGATC ACCACTGAGT CCATTGTGAT CTGGGGCAAG ACCCCCAAGT TCAAGCTGCC CATCCAGAAG GAGACCTGGG AGACCTGGTG GACTGAGTAC TGGCAGGCCA CCTGGATCCC TGAGTGGGAG TTTGTGAACA CCCCCCCT GGTGAAGCTG TGGTACCAGC TGGAGAAGGA GCCCATTGTG GGGGCTGAGA CCTTCTATGT GGCTGGGGCT GCCAACAGGG AGACCAAGCT GGGCAAGGCT 30 GGCTATGTGA CCAACAGGGG CAGGCAGAAG GTGGTGACCC TGACTGACAC CACCAACCAG AAGACTGCCC TCCAGGCCAT CTACCTGGCC CTCCAGGACT CTGGCCTGGA GGTGAACATT GTGACTGCCT CCCAGTATGC CCTGGGCATC ATCCAGGCCC AGCCTGATCA GTCTGAGTCT GTGCCTGCCC ACAAGGGCAT TGGGGGCAAT GAGCAGGTGG ACAAGCTGGT GTCTGCTGGC 35 ATCAGGAAGG TGCTGTTCCT GGATGGCATT GACAAGGCCC AGGATGAGCA TGAGAAGTAC CACTCCAACT GGAGGGCTAT GGCCTCTGAC TTCAACCTGC CCCCTGTGGT GGCTAAGGAG

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ATTGTGGCCT CCTGTGACAA GTGCCAGCTG AAGGGGAGG CCATGCATGG GCAGGTGGAC
TGCTCCCCTG GCATCTGGCA GCTGGCCTGC ACCCACCTGG AGGGCAAGGT GATCCTGGTG
GCTGTGCATG TGGCCTCCGG CTACATTGAG GCTGAGGTGA TCCCTGCTGA GACAGGCCAG
GAGACTGCCT ACTTCCTGCT GAAGCTGGCT GGCAGGTGG CTGTGAAGAC CATCCACACT
GCCAATGGCT CCAACTTCAC TGGGGCCACA GTGAGGGCTG CCTGCTGGTG GGCTGGCATC
AAGCAGGAGT TTGGCATCCC CTACAACCCC CAGTCCCAGG GGGTGGTGGC CTCCATGAAC
AAGGAGCTGA AGAAGATCAT TGGGCAGGTG AGGGACCAGG CTGAGCACCT GAAGACAGCT
GTGCAGATGG CTGTGTTCAT CCACAACTTC AAGAGGAAGG GGGGCATCGG GGGCTACTCC
GCTGGGGAGA GGATTGTGGA CATCATTGCC ACAGACATCC AGACCAAGGA GCTCCAGAAG
CAGATCACCA AGATCCAGAA CTTCAGGGTG TACTACAGGG ACTCCAGGAA CCCCCTGTGG
AAGGGCCCTG CCAAGCTGCT GTGGAAGGGG GAGGGGGCTG TGGTGATCCA GGACAACTCT
GACATCAAGG TGGTGCCCAG GAGGAAGGCC AAGATCATCA GGGACTATGG CAAGCAGATG
GCTGGGGATG ACTGTGTGGC CTCCAGGCAG GATGAGGACT AAAGCCCGGG CAGATCT (SEQ ID
NO:3).

In order to produce the IA-pol-based adenoviral vaccines of the present 15 invention, inactivation of the enzymatic functions was achieved by replacing a total of nine active site residues from the enzyme subunits with alanine side-chains. As shown in Table 1, all residues that comprise the catalytic triad of the polymerase, namely Asp112, Asp187, and Asp188, were substituted with alanine (Ala) residues 20 (Larder, et al., Nature 1987, 327: 716-717; Larder, et al., 1989, Proc. Natl. Acad. Sci. 1989, 86: 4803-4807). Three additional mutations were introduced at Asp445, Glu480 and Asp500 to abolish RNase H activity (Asp551 was left unchanged in this IA Pol construct), with each residue being substituted for an Ala residue, respectively (Davies, et al., 1991, Science 252:, 88-95; Schatz, et al., 1989, FEBS Lett. 257: 311-314; Mizrahi, et al., 1990, Nucl. Acids. Res. 18: pp. 5359-5353). HIV pol integrase 25 function was abolished through three mutations at Asp626, Asp678 and Glu714. Again, each of these residues has been substituted with an Ala residue (Wiskerchen, et al., 1995, J. Virol. 69: 376-386; Leavitt, et al., 1993, J. Biol. Chem. 268: 2113-2119). Amino acid residue Pro3 of SEQ ID NO:4 marks the start of the RT gene. The complete amino acid sequence of IA-Pol is disclosed herein as SEQ ID NO:4 and 30 Figure 17A-C, as follows:

Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg

Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys 10 Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr 15 Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp 20 Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala 25 Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His 35 Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly

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Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val
Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val
Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro
Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu

5 Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr
Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly
Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr
Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn
Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro

10 Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asp
Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp
Glu Asp (SEQ ID NO:4).

As noted above, it will be understood that any combination of the mutations disclosed above may be suitable and therefore be utilized as an IA-pol-based adenoviral HIV vaccine of the present invention, either when administered alone or in a combined modality regime and/or a prime-boost regimen. For example, it may be possible to mutate only 2 of the 3 residues within the respective reverse transcriptase, RNase-H, and integrase coding regions while still abolishing these enzymatic activities. However, the IA-pol construct described above and disclosed as SEQ ID NO:3, as well as the expressed protein (SEQ ID NO:4;) is preferred. It is also preferred that at least one mutation be present in each of the three catalytic domains.

Another aspect of this portion of the invention are codon optimized HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA (tissue-type plasminogen activator) or by a leader peptide such as is found in highly expressed mammalian proteins such as immunoglobulin leader peptides. Any functional leader peptide may be tested for efficacy. However, a preferred embodiment of the present invention, as with HIV-1 Nef constructs shown herein, is to provide for a HIV-1 Pol mutant adenoviral vaccine construction wherein the pol coding region or a portion thereof is operatively linked to a leader peptide, preferably a leader peptide from human tPA. In other words, a codon optimized HIV-1 Pol mutant such as IA-Pol (SEQ ID NO:4) may also comprise a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. As noted in Figure 16A-B, a DNA vector which may be utilized to practice the present invention may be modified by known recombinant DNA methodology to contain a leader signal

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peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Pol protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Pol protein of interest. Regardless of the cloning strategy, the end result. is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Pol protein of interest, including but not limited to a HIV-1 Pol protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17). Therefore, another aspect of the present invention is to generate HIV-1 Pol-based vaccine constructions which comprise a eukaryotic trafficking signal peptide such as from tPA. To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5' end of the coding region. A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame disclosed herein as SEQ ID NO:6.

To this end, the present invention relates to a DNA molecule which encodes a codon optimized wt-pol DNA construct wherein the protease (PR) activity is deleted and a human tPA leader sequence is fused to the 5'end of the coding region (herein, "tPA-wt-pol"). A DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:5, the open reading frame being contained from an initiating Met residue at nucleotides 8-10 to a termination codon from nucleotides 2633-2635. SEQ ID NO:5 is as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT
CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA
GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT
CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG
CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG
GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA
GCTGGGCATCC CCCCACCCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGATGT
GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC
CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA
GGGCTGGAAG GGCTCCCCTG CCATCTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT
CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GATGACCTGT ATGTGGCTC
TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAC



The open reading frame of the wild type tPA-pol construct disclosed as SEQ ID NO:5 contains 875 amino acids, disclosed herein as SEQ ID NO:6, as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly

Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Asp Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro 10 Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Asp Asp Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp 15 Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile 20 Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln 25 Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Asp Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu 30 Thr Asp Thr Thr Asn Gln Lys Thr.Glu Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Asp Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu 35 Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile

Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Asp Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Asp Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly 10 Val Val Glu Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp 15 Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:6).

The present invention also relates to a codon optimized HIV-1 Pol mutant 20 contained within a recombinant adenoviral vector such as IA-Pol (SEQ ID NO:4) which comprises a leader peptide at the amino terminal portion of the protein, which may effect cellular trafficking and hence, immunogenicity of the expressed protein within the host cell. Any such adenoviral-based HIV-1 DNA pol mutant disclosed in the above paragraphs is suitable for fusion downstream of a leader peptide, such as a 25 leader peptide including but not limited to the human tPA leader sequence. Therefore, any such leader peptide-based HIV-1 pol mutant construct may include but is not limited to a mutated DNA molecule which effectively alters the catalytic activity of the RT, RNase and/or IN region of the expressed protein, resulting in at least substantially decreased enzymatic activity one or more of the RT, RNase H and/or IN 30 functions of HIV-1 Pol. In a preferred embodiment of this portion of the invention, a leader peptide/HIV-1 DNA pol construct contains a mutation or mutations within the Pol coding region which effectively abolishes RT, RNase H and IN activity. An especially preferable HIV-1 DNA pol construct is a DNA molecule which contains at least one point mutation which alters the active site and catalytic activity within the 35 RT, RNase H and IN domains of Pol, such that each activity is at least substantially abolished, and preferably totally abolished. Such a HIV-1 Pol mutant will most likely

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comprise at least one point mutation in or around each catalytic domain responsible for RT, RNase H and IN activity, respectfully. An especially preferred embodiment of this portion of the invention relates to a human tPA leader fused to the IA-Pol protein comprising the nine mutations shown in Table 1. The DNA molecule is disclosed herein as SEQ ID NO:7 and the expressed tPA-IA Pol protein comprises a fusion junction as shown in Figure 18. The complete amino acid sequence of the expressed protein is set forth in SEQ ID NO:8. To this end, SEQ ID NO:7 discloses the nucleotide sequence which codes for a human tPA leader fused to the IA Pol protein comprising the nine mutations shown in Table 1 (herein, "tPA-opt-IApol"). The open 10 reading frame begins with the initiating Met (nucleotides 8-10) and terminates with a "TAA" codon at nucleotides 2633-2635. The nucleotide sequence encoding tPA-IAPol is also disclosed as follows:

GATCACCATG GATGCAATGA AGAGAGGGCT CTGCTGTGTG CTGCTGCTGT GTGGAGCAGT CTTCGTTTCG CCCAGCGAGA TCTCCGCCCC CATCTCCCCC ATTGAGACTG TGCCTGTGAA GCTGAAGCCT GGCATGGATG GCCCCAAGGT GAAGCAGTGG CCCCTGACTG AGGAGAAGAT CAAGGCCCTG GTGGAAATCT GCACTGAGAT GGAGAAGGAG GGCAAAATCT CCAAGATTGG CCCCGAGAAC CCCTACAACA CCCCTGTGTT TGCCATCAAG AAGAAGGACT CCACCAAGTG GAGGAAGCTG GTGGACTTCA GGGAGCTGAA CAAGAGGACC CAGGACTTCT GGGAGGTGCA GCTGGGCATC CCCCACCCG CTGGCCTGAA GAAGAAGAAG TCTGTGACTG TGCTGGCTGT GGGGGATGCC TACTTCTCTG TGCCCCTGGA TGAGGACTTC AGGAAGTACA CTGCCTTCAC CATCCCCTCC ATCAACAATG AGACCCCTGG CATCAGGTAC CAGTACAATG TGCTGCCCCA GGGCTGGAAG GGCTCCCCTG CCATCTTCCA GTCCTCCATG ACCAAGATCC TGGAGCCCTT CAGGAAGCAG AACCCTGACA TTGTGATCTA CCAGTACATG GCTGCCCTGT ATGTGGGCTC TGACCTGGAG ATTGGGCAGC ACAGGACCAA GATTGAGGAG CTGAGGCAGC ACCTGCTGAG GTGGGGCCTG ACCACCCCTG ACAAGAAGCA CCAGAAGGAG CCCCCCTTCC TGTGGATGGG CTATGAGCTG CACCCCGACA AGTGGACTGT GCAGCCCATT GTGCTGCCTG AGAAGGACTC CTGGACTGTG AATGACATCC AGAAGCTGGT GGGCAAGCTG AACTGGGCCT CCCAAATCTA CCCTGGCATC AAGGTGAGGC AGCTGTGCAA GCTGCTGAGG GGCACCAAGG CCCTGACTGA GGTGATCCCC CTGACTGAGG AGGCTGAGCT GGAGCTGGCT GAGAACAGGG AGATCCTGAA GGAGCCTGTG CATGGGGTGT ACTATGACCC CTCCAAGGAC CTGATTGCTG AGATCCAGAA -GCAGGGCCAG GGCCAGTGGA CCTACCAAAT CTACCAGGAG CCCTTCAAGA ACCTGAAGAC TGGCAAGTAT GCCAGGATGA GGGGGGCCCA CACCAATGAT GTGAAGCAGC TGACTGAGGC TGTGCAGAAG ATCACCACTG AGTCCATTGT GATCTGGGGC AAGACCCCCA AGTTCAAGCT GCCCATCCAG AAGGAGACCT GGGAGACCTG GTGGACTGAG TACTGGCAGG CCACCTGGAT CCCTGAGTGG GAGTTTGTGA ACACCCCCC CCTGGTGAAG CTGTGGTACC AGCTGGAGAA GGAGCCCATT GTGGGGGCTG AGACCTTCTA TGTGGCTGGG GCTGCCAACA GGGAGACCAA

GCTGGCCAG GCTGGCTATG TGACCAACAG GGGCAGGCAG AAGGTGGTGA CCCTGACTGA CACCACCAAC CAGAAGACTG CCCTCCAGGC CATCTACCTG GCCCTCCAGG ACTCTGGCCT GGAGGTGAAC ATTGTGACTG CCTCCCAGTA TGCCCTGGGC ATCATCCAGG CCCAGCCTGA TCAGTCTGAG TCTGAGCTGG TGAACCAGAT CATTGAGCAG CTGATCAAGA AGGAGAAGGT GTACCTGGCC TGGGTGCCTG CCCACAAGGG CATTGGGGGC AATGAGCAGG TGGACAAGCT GGTGTCTGCT GGCATCAGGA AGGTGCTGTT CCTGGATGGC ATTGACAAGG CCCAGGATGA GCATGAGAAG TACCACTCCA ACTGGAGGGC TATGGCCTCT GACTTCAACC TGCCCCCTGT GGTGGCTAAG GAGATTGTGG CCTCCTGTGA CAAGTGCCAG CTGAAGGGGG AGGCCATGCA TGGGCAGGTG GACTGCTCCC CTGGCATCTG GCAGCTGGCC TGCACCCACC TGGAGGGCAA 10 GGTGATCCTG GTGGCTGTGC ATGTGGCCTC CGGCTACATT GAGGCTGAGG TGATCCCTGC TGAGACAGGC CAGGAGACTG CCTACTTCCT GCTGAAGCTG GCTGGCAGGT GGCCTGTGAA GACCATCCAC ACTGCCAATG GCTCCAACTT CACTGGGGCC ACAGTGAGGG CTGCCTGCTG GTGGGCTGGC ATCAAGCAGG AGTTTGGCAT CCCCTACAAC CCCCAGTCCC AGGGGGTGGT GGCCTCCATG AACAAGGAGC TGAAGAAGAT CATTGGGCAG GTGAGGGACC AGGCTGAGCA CCTGAAGACA GCTGTGCAGA TGGCTGTGTT CATCCACAAC TTCAAGAGGA AGGGGGGCAT 15 CGGGGGCTAC TCCGCTGGGG AGAGGATTGT GGACATCATT GCCACAGACA TCCAGACCAA GGAGCTCCAG AAGCAGATCA CCAAGATCCA GAACTTCAGG GTGTACTACA GGGACTCCAG GAACCCCTG TGGAAGGGCC CTGCCAAGCT GCTGTGGAAG GGGGAGGGGG CTGTGGTGAT CCAGGACAAC TCTGACATCA AGGTGGTGCC CAGGAGGAAG GCCAAGATCA TCAGGGACTA TGGCAAGCAG ATGGCTGGGG ATGACTGTGT GGCCTCCAGG CAGGATGAGG ACTAAAGCCC 20 GGGCAGATCT (SEQ ID NO:7).

The open reading frame of the tPA-IA-pol construct disclosed as SEQ ID NO:7 contains 875 amino acids, disclosed herein as tPA-IA-Pol and SEQ ID NO:8, as follows:

25 Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu 30 Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr

Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro Asp Lys Trp Thr Val Gln Pro Ile Val 5 Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu 10 Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe 15 Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala 20 Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp 25 Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val Asp Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu 30 Ala Glu Val Ile Pro Ala Glu Thr Gly Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val 35 Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe

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Ile His Asn Phe Lys Arg Lys Gly Gly Ile Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp (SEQ ID NO:8).

EXAMPLE 18

CODON OPTIMIZED HIV-1 NEF AND CODON OPTIMIZED HIV-1 NEF MODIFICATIONS

Codon optimized version of HIV-1 Nef and HIV-1 Nef modifications are essentially as described in U.S. Application Serial No. 09/738,782, filed December 15, 2000 and PCT International Application PCT/US00/34162, also filed December 15, 2000, both documents which are hereby incorporated by reference. As disclosed within the above-mentioned documents, particular embodiments of codon optimized Nef and Nef modifications relate to a DNA molecule encoding HIV-1 Nef from the HTV-1 ifrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:9, while the expressed open reading frame is disclosed herein as SEQ ID NO:10. Another embodiment of Nef-based coding regions for use in the adenoviral vectors of the present invention comprise a codon optimized DNA molecule encoding a protein containing the human plasminogen activator (tpa) leader peptide fused with the NH₂-terminus of the HIV-1 Nef polypeptide. The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:11, while the expressed open reading frame is disclosed herein as SEQ ID NO:12. Another modified Nef optimized coding region relates to a DNA molecule encoding optimized HIV-1 Nef wherein the open reading frame codes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175, herein described as opt nef (G2A, LLAA). The DNA molecule which encodes this protein is disclosed herein as SEQ ID NO:13, while the expressed open reading frame is disclosed herein as SEQ ID NO:14. An additional embodiment relates to a DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA), comprises an open reading frame which

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AAAGCCCGGG C (SEQ ID NO:9).

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encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175, herein referred to as opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, while the expressed open reading frame is disclosed herein as SEQ ID NO:16.

As disclosed in the above-identified documents (U.S. Application Serial No. 09/738,782 and PCT International Application PCT/US00/34162) and reiterated herein, the following nef-based nucleotide and amino acid sequences which comprise the respective open reading frame are as follows:

The nucleotide sequence of the codon optimized version of HIV-1 jrfl

nef gene is disclosed herein as SEQ ID NO:9, as shown herein: GATCTGCCAC CATGGGCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT ACACCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC CTGCTGCACC CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT

CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT

Preferred codon usage is as follows: Met (ATG), Gly (GGC), Lys (AAG), Trp (TGG), Ser (TCC), Arg (AGG), Val (GTG), Pro (CCC), Thr (ACC), Glu (GAG); Leu (CTG), His (CAC), Ile (ATC), Asn (AAC), Cys (TGC), Ala (GCC), Gln (CAG), Phe (TTC) and Tyr (TAC). For an additional discussion relating to mammalian (human) codon optimization, see WO 97/31115 (PCT/US97/02294), which is hereby incorporated by reference. See also Figure 19A-B for a comparion of wild type vs. codon optimized nucleotides comprising the open reading frame of HIV-Nef.

The open reading frame for SEQ ID NO:9 above comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The open reading frame of SEQ ID NO:9 provides for a 216 amino acid HTV-1 Nef protein expressed through utilization of a codon optimized DNA vaccine vector. The 216 amino acid HIV-1 Nef (jfrl) protein is disclosed herein as SEQ ID 35 NO:10, and as follows:

Met Gly Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val

Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:10).

HIV-1 Nef is a 216 amino acid cytosolic protein which associates with the inner surface of the host cell plasma membrane through myristylation of Gly-2 15 (Franchini et al., 1986, Virology 155: 593-599). While not all possible Nef functions have been elucidated, it has become clear that correct trafficking of Nef to the inner plasma membrane promotes viral replication by altering the host intracellular environment to facilitate the early phase of the HIV-1 life cycle and by increasing the infectivity of progeny viral particles. In one aspect of the invention regarding 20 codon-optimized, protein-modified polypeptides, the nef-encoding region of the adenovirus vector of the present invention is modified to contain a nucleotide sequence which encodes a heterologous leader peptide such that the amino terminal region of the expressed protein will contain the leader peptide. The diversity of function that typifies eukaryotic cells depends upon the structural differentiation of 25 their membrane boundaries. To generate and maintain these structures, proteins must be transported from their site of synthesis in the endoplasmic reticulum to predetermined destinations throughout the cell. This requires that the trafficking proteins display sorting signals that are recognized by the molecular machinery responsible for route selection located at the access points to the main trafficking 30 pathways. Sorting decisions for most proteins need to be made only once as they traverse their biosynthetic pathways since their final destination, the cellular location at which they perform their function, becomes their permanent residence. Maintenance of intracellular integrity depends in part on the selective sorting and accurate transport of proteins to their correct destinations. Defined sequence motifs 35 exist in proteins which can act as 'address labels'. A number of sorting signals have

been found associated with the cytoplasmic domains of membrane proteins. An effective induction of CTL responses often required sustained, high level endogenous expression of an antigen. As membrane-association via myristylation is an essential requirement for most of Nef's function, mutants lacking myristylation, by glycine-to-alanine change, change of the dileucine motif and/or by substitution with a tpa leader sequence as described herein, will be functionally defective, and therefore will have improved safety profile compared to wild-type Nef for use as an HIV-1 vaccine component.

In another embodiment of this portion of the invention, either the DNA vector or the HIV-1 nef nucleotide sequence is modified to include the human tissue-specific plasminogen activator (tPA) leader. As shown in Figure 16A-B, a DNA vector may be modified by known recombinant DNA methodology to contain a leader signal peptide of interest, such that downstream cloning of the modified HIV-1 protein of interest results in a nucleotide sequence which encodes a modified HIV-1 tPA/Nef protein. In the alternative, as noted above, insertion of a nucleotide sequence which encodes a leader peptide may be inserted into a DNA vector housing the open reading frame for the Nef protein of interest. Regardless of the cloning strategy, the end result is a polynucleotide vaccine which comprises vector components for effective gene expression in conjunction with nucleotide sequences which encode a modified HIV-1 Nef protein of interest, including but not limited to a HIV-1 Nef protein which contains a leader peptide. The amino acid sequence of the human tPA leader utilized herein is as follows: MDAMKRGLCCVLLLCGAVFVSPSEISS (SEQ ID NO:17).

It has been shown that myristylation of Gly-2 in conjunction with a dileucine motif in the carboxy region of the protein is essential for Nef-induced down regulation of CD4 (Aiken et al., 1994, Cell 76: 853-864) via endocytosis. It has also been shown that Nef expression promotes down regulation of MHCI (Schwartz et al., 1996, Nature Medicine 2(3): 338-342) via endocytosis. The present invention relates in part to DNA vaccines which encode modified Nef proteins altered in trafficking and/or functional properties. The modifications introduced into the adenoviral vector HIV vaccines of the present invention include but are not limited to additions, deletions or substitutions to the nef open reading frame which results in the expression of a modified Nef protein which includes an amino terminal leader peptide, modification or deletion of the amino terminal myristylation site, and modification or deletion of the dileucine motif within the Nef protein and which alter function within the infected host cell. Therefore, a central theme of the DNA molecules and recombinant adenoviral HIV vaccines of the present invention is (1)

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host administration and intracellular delivery of a codon optimized nef-based adenoviral HIV vaccine; (2) expression of a modified Nef protein which is immunogenic in terms of eliciting both CTL and Th responses; and, (3) inhibiting or at least altering known early viral functions of Nef which have been shown to promote HIV-1 replication and load within an infected host. Therefore, the nef coding region may be altered, resulting in a DNA vaccine which expresses a modified Nef protein wherein the amino terminal Gly-2 myristylation residue is either deleted or modified to express alternate amino acid residues. Also, the nef coding region may be altered so as to result in a DNA vaccine which expresses a modified Nef protein wherein the dileucine motif is either deleted or modified to express alternate amino acid residues. In addition, the adenoviral vector HIV vaccines of the present invention also relate to an isolated DNA molecule, regardless of codon usage, which expresses a wild type or modified Nef protein as described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion or substitution of Leu 174 and Leu 175 and/or inclusion of a leader sequence.

Therefore, specific Nef-based constructs further include the following, as exemplification's and not limitations. For example, the present invention relates to an adenoviral vector vaccine which encodes modified forms of HIV-1, an open reading frame which encodes a Nef protein which comprises a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl) is referred to herein as opt tpanef. The nucleotide sequence comprising the open reading frame of opt tpanef is disclosed herein as SEQ ID NO:11, as shown below:

CATGGATGCA ATGAAGAGA GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAG CCAACGAGGG CGAGAACAAC TGCCTGCTGC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACCCCATGTC
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCC
(SEQ ID NO:11).

The open reading frame for SEQ ID NO:11 comprises an initiating methionine

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residue at nucleotides 2-4 and a "TAA" stop codon from nucleotides 713-715. The open reading frame of SEQ ID NO:3 provides for a 237 amino acid HIV-1 Nef protein which comprises a tPA leader sequence fused to amino acids 6-216 of HIV-1 Nef, including the dileucine motif at amino acid residues 174 and 175. This 237 amino acid tPA/Nef (jfrl) fusion protein is disclosed herein as SEQ ID NO:12, and is shown as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val 10 Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp 15 Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Leu Leu His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu 20 Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:12). Therefore, this exemplified Nef protein, Opt tPA-Nef, contains both a tPA leader sequence as well as deleting the myristylation site of Gly-2A DNA molecule encoding HIV-1 Nef from the HIV-1 jfrl isolate wherein the codons are optimized for expression in a mammalian system such as a human. 25

In another specific embodiment of the present invention, a DNA molecule is disclosed which encodes optimized HIV-1 Nef wherein the open reading frame of a recombinant adenoviral HIV vaccine encodes for modifications at the amino terminal myristylation site (Gly-2 to Ala-2) and substitution of the Leu-174-Leu-175 dileucine motif to Ala-174-Ala-175. This open reading frame is herein described as opt nef (G2A,LLAA) and is disclosed as SEQ ID NO:13, which comprises an initiating methionine residue at nucleotides 12-14 and a "TAA" stop codon from nucleotides 660-662. The nucleotide sequence of this codon optimized version of HIV-1 jrfl nef gene with the above mentioned modifications is disclosed herein as SEQ ID NO:13, as follows:

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GATCTGCCAC CATGGCCGGC AAGTGGTCCA AGAGGTCCGT GCCCGGCTGG TCCACCGTGA
GGGAGAGGAT GAGGAGGGCC GAGCCCGCCG CCGACAGGGT GAGGAGGACC GAGCCCGCCG
CCGTGGGCGT GGGCGCCGTG TCCAGGGACC TGGAGAAGCA CGGCGCCATC ACCTCCTCCA
ACACCGCCGC CACCAACGCC GACTGCGCCT GGCTGGAGGC CCAGGAGGAC GAGGAGGTGG
GCTTCCCCGT GAGGCCCCAG GTGCCCCTGA GGCCCATGAC CTACAAGGGC GCCGTGGACC
TGTCCCACTT CCTGAAGGAG AAGGGCGGCC TGGAGGGCCT GATCCACTCC CAGAAGAGGC
AGGACATCCT GGACCTGTGG GTGTACCACA CCCAGGGCTA CTTCCCCGAC TGGCAGAACT
ACACCCCCGG CCCCGGCATC AGGTTCCCCC TGACCTTCGG CTGGTGCTTC AAGCTGGTGC
CCGTGGAGCC CGAGAAGGTG GAGGAGGCCA ACGAGGGCGA GAACAACTGC GCCGCCCACC
CCATGTCCCA GCACGGCATC GAGGACCCCG AGAAGGAGGT GCTGGAGTGG AGGTTCGACT
CCAAGCTGGC CTTCCACCAC GTGGCCAGGG AGCTGCACCC CGAGTACTAC AAGGACTGCT
AAAGCCCGGG C (SEQ ID NO:13).

The open reading frame of SEQ ID NO:13 encodes Nef (G2A,LLAA), disclosed herein as SEQ ID NO:14, as follows:

Met Ala Gly Lys Trp Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val 15 Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr Lys Gly Ala Val Asp 20 Leu Ser His Phe Leu Lys Glu Lys Gly Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn Asn Cys Ala Ala His 25 Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys Ser (SEQ ID NO:14).

An additional embodiment of the present invention relates to another DNA molecule encoding optimized HIV-1 Nef wherein the amino terminal myristylation site and dileucine motif have been deleted, as well as comprising a tPA leader peptide. This DNA molecule, opt tpanef (LLAA) comprises an open reading frame which encodes a Nef protein containing a tPA leader sequence fused to amino acid residue 6-216 of HIV-1 Nef (jfrl), wherein Leu-174 and Leu-175 are substituted with Ala-174 and Ala-175 (Ala-195 and Ala-196 in this tPA-based fusion protein). The nucleotide

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sequence comprising the open reading frame of opt tpanef (LLAA) is disclosed herein as SEQ ID NO:15, as shown below:

CATGGATGCA ATGAAGAGAG GGCTCTGCTG TGTGCTGCTG CTGTGTGGAG CAGTCTTCGT
TTCGCCCAGC GAGATCTCCT CCAAGAGGTC CGTGCCCGGC TGGTCCACCG TGAGGGAGAG
GATGAGGAGG GCCGAGCCCG CCGCCGACAG GGTGAGGAGG ACCGAGCCCG CCGCCGTGGG
CGTGGGCGCC GTGTCCAGGG ACCTGGAGAA GCACGGCGCC ATCACCTCCT CCAACACCGC
CGCCACCAAC GCCGACTGCG CCTGGCTGGA GGCCCAGGAG GACGAGGAGG TGGGCTTCCC
CGTGAGGCCC CAGGTGCCCC TGAGGCCCAT GACCTACAAG GGCGCCGTGG ACCTGTCCCA
CTTCCTGAAG GAGAAGGGCG GCCTGGAGGG CCTGATCCAC TCCCAGAAGA GGCAGGACAT
CCTGGACCTG TGGGTGTACC ACACCCAGGG CTACTTCCCC GACTGGCAGA ACTACACCCC
CGGCCCCGGC ATCAGGTTCC CCCTGACCTT CGGCTGGTGC TTCAAGCTGG TGCCCGTGGA
GCCCGAGAAG GTGGAGGAGG CCAACGAGGG CGAGAACAAC TGCGCCGCCC ACCCCATGTC
CCAGCACGGC ATCGAGGACC CCGAGAAGGA GGTGCTGGAG TGGAGGTTCG ACTCCAAGCT
GGCCTTCCAC CACGTGGCCA GGGAGCTGCA CCCCGAGTAC TACAAGGACT GCTAAAGCCC
(SEQ ID NO:15).

The open reading frame of SEQ ID NO:7 encoding tPA-Nef (LLAA), disclosed herein as SEQ ID NO:16, is as follows:

Met Asp Ala Met Lys Arg Gly Leu Cys Cys Val Leu Leu Leu Cys Gly Ala Val Phe Val Ser Pro Ser Glu Ile Ser Ser Lys Arg Ser Val Pro Gly Trp Ser Thr Val Arg Glu Arg Met Arg Arg Ala Glu Pro Ala Ala Asp Arg Val Arg Arg Thr Glu Pro Ala Ala Val Gly Val Gly Ala Val Ser Arg Asp Leu Glu Lys His Gly Ala Ile Thr Ser Ser Asn Thr Ala Ala Thr Asn Ala Asp Cys Ala Trp Leu Glu Ala Gln Glu Asp Glu Glu Val Gly Phe Pro Val Arg Pro Gln Val Pro Leu Arg Pro Met Thr Tyr 25 Lys Gly Ala Val Asp Leu Ser His Phe Leu Lys Glu Lys Gly Leu Glu Gly Leu Ile His Ser Gln Lys Arg Gln Asp Ile Leu Asp Leu Trp Val Tyr His Thr Gln Gly Tyr Phe Pro Asp Trp Gln Asn Tyr Thr Pro Gly Pro Gly Ile Arg Phe Pro Leu Thr Phe Gly Trp Cys Phe Lys Leu Val Pro Val Glu Pro Glu Lys Val Glu Glu Ala Asn Glu Gly Glu Asn 30 Asn Cys Ala Ala His Pro Met Ser Gln His Gly Ile Glu Asp Pro Glu Lys Glu Val Leu Glu Trp Arg Phe Asp Ser Lys Leu Ala Phe His His Val Ala Arg Glu Leu His Pro Glu Tyr Tyr Lys Asp Cys (SEQ ID NO:16). An adenoviral vector of the present invention may comprise a DNA sequence, regardless of codon usage, which expresses a wild type or modified Nef protein as 35 described herein, including but not limited to modified Nef proteins which comprise a deletion or substitution of Gly 2, a deletion of substitution of Leu 174 and Leu 175

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and/or inclusion of a leader sequence. Therefore, partial or fully codon optimized DNA vaccine expression vector constructs are preferred since such constructs should result in increased host expression. However, it is within the scope of the present invention to utilize "non-codon optimized" versions of the constructs disclosed herein, especially modified versions of HIV Nef which are shown to promote a substantial cellular immune response subsequent to host administration.

Figure 20A-C show nucleotide sequences at junctions between nef coding sequence and plasmid backbone of nef expression vectors V1Jns/nef (Figure 20A), V1Jns/nef(G2A,LLAA) (Figure 20B), V1Jns/tpanef (Figure 20C) and V1Jns/tpanef(LLAA) (Figure 20C, also). 5' and 3' flanking sequences of codon optimized nef or codon optimized nef mutant genes are indicated by bold/italic letters; nef and nef mutant coding sequences are indicated by plain letters. Also indicated (as underlined) are the restriction endonuclease sites involved in construction of respective nef expression vectors. V1Jns/tpanef and V1Jns/tpanef(LLAA) have identical sequences at the junctions.

Figure 21 shows a schematic presentation of nef and nef derivatives. Amino acid residues involved in Nef derivatives are presented. Glycine 2 and Leucine 174 and 175 are the sites involved in myristylation and dileucine motif, respectively.

20 EXAMPLE 19

MRKAd5Pol Construction and Virus Rescue

Steps performed in the construction of the vectors, including the pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus plasmid denoted MRKAd5pol, is depicted in Figure 22. Briefly, the adenoviral shuttle vector for the full-length inactivated HIV-1 pol gene is as follows. The vector MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is a derivative of the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. The vector contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique BglII site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 (or MRKpAdHVE3) preplasmid. The vector, similar to the original shuttle vector contains the Pac1 site, extension to the packaging signal region, and extension to the pIX gene. The synthetic full-length codon-optimized HIV-1 pol gene was isolated directly from the plasmid pV1Ins-HIV-pol-inact(opt). Digestion of this plasmid with Bgl II releases the pol

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gene intact (comprising a codon optimized IA pol sequence as disclosed in SEQ ID NO:3). The pol fragment was gel purified and ligated into the MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) shuttle vector at the BgIII site. The clones were checked for the correct orientation of the gene by using restriction enzymes DraIII/Not1. A positive clone was isolated and named MRKpdel+hCMVmin+FL-pol+bGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdel+hCMVmin+FLpol+bGHpA(S) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1-/E3+)Cla1. The resulting pre-plasmid originally named MRKpAd+hCMVmin+FLpol+bGHpA(S)E3+ is now referred to as "pMRKAd5pol". The genetic structure of the resulting pMRKAd5pol was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the pol transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1pol adenoviral vector is shown in Figure 26 A-AO.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5pol, was rescued as infectious virions in PER.C6® adherent monolayer cell culture. To rescue infectious virus, 12 μ g of pMRKAd5pol was digested with restriction enzyme PacI (New England Biolabs) and 3.3 μ g was transfected per 6 cm dish of PER.C6® cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech Inc.). PacI digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6® cells. Infected cells and media were harvested 6 -10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This pol containing recombinant adenovirus is referred to herein as "MRKAd5pol". This recombinant adenovirus expresses an inactivated HIV-1 Pol protein as shown in SEQ ID NO:6.

EXAMPLE 20

MRKAd5Nef Construction and Virus Rescue

Construction of vector: shuttle plasmid and pre-adenovirus plasmid - Key steps performed in the construction of the vectors, including the pre-adenovirus

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plasmid denoted MRKAd5nef, is depicted in Figure 23. Briefly, as shown in Example 19 above, the vector

MRKpdelE1(Pac/pIX/pack450)+CMVmin+BGHpA(str.) is the shuttle vector used in the construction of the MRKAd5gag adenoviral pre-plasmid. It has been modified to contain the *Pac*1 site, extension to the packaging signal region, and extension to the pIX gene. It contains an expression cassette with the hCMV promoter (no intronA) and the bovine growth hormone polyadenylation signal. The expression unit has been inserted into the shuttle vector such that insertion of the gene of choice at a unique *Bgl*11 site will ensure the direction of transcription of the transgene will be Ad5 E1 parallel when inserted into the MRKpAd5(E1-/E3+)Cla1 pre-plasmid. The synthetic full-length codon-optimized HIV-1 nef gene was isolated directly from the plasmid pV1Jns/nef (G2A,LLAA). Digestion of this plasmid with *Bgl*11 releases the pol gene intact, which comprises the nucleotide sequence as disclosed in SEQ ID NO:13. The nef fragment was gel purified and ligated into the

MRKpdelE1+CMVmin+BGHpA(str.) shuttle vector at the Bgl11 site. The clones were checked for correction orientation of the gene by using restriction enzyme Scal. A positive clone was isolated and named MRKpdelE1hCMVminFL-nefBGHpA(s). The genetic structure of this plasmid was verified by PCR, restriction enzyme and DNA sequencing. The pre-adenovirus plasmid was constructed as follows. Shuttle plasmid MRKpdelE1hCMVminFL-nefBGHpA(s) was digested with restriction enzymes Pac1 and Bst1107 I (or its isoschizomer, BstZ107 I) and then co-transformed into E. coli strain BJ5183 with linearized (Cla1 digested) adenoviral backbone plasmid, MRKpAd(E1/E3+)Cla1. The resulting pre-plasmid originally named MRKpdelE1hCMVminFL-nefBGHpA(s) is now referred to as "pMRKAd5nef". The genetic structure of the resulting pMRKAd5nef was verified by PCR, restriction enzyme and DNA sequence analysis. The vectors were transformed into competent E. coli XL-1 Blue for preparative production. The recovered plasmid was verified by restriction enzyme digestion and DNA sequence analysis, and by expression of the nef transgene in transient transfection cell culture. The complete nucleotide sequence of this pMRKAd5HIV-1nef adenoviral vector is shown in Figure 27A-AM.

Generation of research-grade recombinant adenovirus - The pre-adenovirus plasmid, pMRKAd5nef, was rescued as infectious virions in PER.C6[®] adherent monolayer cell culture. To rescue infectious virus, 12 μg of pMRKAdnef was digested with restriction enzyme Pac1 (New England Biolabs) and 3.3 μg was transfected per 6 cm dish of PER.C6[®] cells using the calcium phosphate coprecipitation technique (Cell Phect Transfection Kit, Amersham Pharmacia Biotech

Inc.). Pac1 digestion releases the viral genome from plasmid sequences allowing viral replication to occur after entry into PER.C6®cells. Infected cells and media were harvested 6-10 days post-transfection, after complete viral cytopathic effect (CPE) was observed. Infected cells and media were stored at \leq -60°C. This nef containing recombinant adenovirus is now referred to as "MRKAd5nef".

EXAMPLE 21

Construction of Murine CMV Promoter Containing Shuttle Vectors for Inactivated Pol and Nef/G2A,LLAA

The murine CMV (mCMV) was amplified from the plasmid pMH4 (supplied 10 by Frank Graham, McMaster University) using the primer set: mCMV (Not I) Forward: 5'-ATA AGA ATG CGG CCG CCA TAT ACT GAG TCA TTA GG-3' (SEO ID NO: 20); mCMV (Bgl II)Reverse: 5'-AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C-3' (SEQ ID NO:21). The underlined nucleotides represent the Not I and the Bgl II sites respectively for each primer. This PCR amplicon was 15 used for the construction of the mCMV shuttle vector containing the transgene in the El parallel orientation. The hCMV promoter was removed from the original shuttle vector (containing the hCMV-gag-bGHpA transgene in the E1 parallel orientation) by digestion with Not I and Bgl II. The mCMV promoter (Not I/Bgl II digested PCR product) was inserted into the shuttle vector in a directional manner. The shuttle 20 vector was then digested with Bgl II and the gag reporter gene (Bgl II fragment) was re-inserted back into the shuttle vector. Several clones were screened for correct orientation of the reporter gene. For the construction of the mCMV-gag in the E1 antiparallel orientation, the mCMV promoter was amplified from the plasmid pMH4 using the following primer set: mCMV (Asc I) Forward: 5'- ATA AGA ATG GCG 25 CGC CAT ATA CTG AGT CAT TAG G (SEQ ID NO:22); mCMV (Bgl II) Reverse: 5' AAG GAA GAT CTA CCG ACG CTG GTC GCG CCT C (SEQ ID NO:23). The underlined nucleotides represent the Asc I and Bgl II sites, respectively for each primer. The shuttle vector containing the hCMV-gag transgene in the E1 antiparallel orientation was digested with Asc1 and Bgl11 to remove the hCMV-gag portion of the 30 transgene. The mCMV promoter (Asc1/Bgl11 digested PCR product) was inserted into the shuttle vector in a directional manner. The vector was then digested with Bgl11 and the gag reporter gene (Bgl11 fragment) was re-inserted. Several clones were screened for correct orientation of the reporter gene. For each of the full length IA pol and full length nef/G2A,LLAA genes, cloning was performed using the unique 35

 $Bgl ext{ II}$ site within the mCMV-bGHpA shuttle vector. The pol and nef genes were excised from their respective pV1Jns plasmids by $Bgl ext{ II}$ digestion.

EXAMPLE 22

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Construction of mCMV Full Length Inactivated Pol and Full Length nef/G2A.LLAA Adenovectors

Each of these transgenes of Example 21 were inserted into the modified shuttle vector in both the E1 parallel and E1 anti-parallel orientations. *Pac1* and *BstZ110I* digestion of each shuttle vector was performed and each specific transgene fragment containing the flanking Ad5 sequences was isolated and co-transformed with *Cla* I digested MRKpAd5(E3+) or MRKpAd5(E3-) adenovector plasmids via bacterial homologous recombination in BJ5183 *E. coli* cells. Recombinant preplasmid adenovectors containing the various transgenes in both the E3- and E3+ versions (and in the E1 parallel and E1 antiparallel orientations) were subsequently prepared in large scale following transformation into XL-1 Blue *E. coli* cells and analyzed by restriction analysis and sequencing.

EXAMPLE 23

Construction of hCMV-tpa-nef (LLAA) Adenovector

The tpa-nef gene was amplified out from GMP grade pV1Jns-tpanef (LLAA) vector using the primer sets: Tpanef (BamHI) F 5'-ATT GGA TCC ATG GAT GCA ATG AAG AGA GGG (SEQ ID 24); Tpanef (BamHI) R 5'-ATA GGA TCC TTA GCA GTC CTT GTA GTA CTC G (SEQ ID NO:25). The resulting PCR product was digested with BamHI, gel purified and cloned into the Bgl II site of MRKAd5CMV-bGHpA shuttle vector (Bgl II digested and calf intestinal phosphatase treated). Clones containing the tpanef (LLAA) gene (see SEQ ID NO:15 for complet coding region) in the correct orientation with respect to the hCMV promoter were selected following Sca I digestion. The resulting MRKAd5tpanef shuttle vector was digested with Pac I and Bst Z1101 and cloned into the E3+ MRKAd5 adenovector via bacterial homologous recombination techniques.

EXAMPLE 24

Immunogenicity of MRKAd5pol and MRKAd5nef Vaccine

Materials and Methods - Rodent Immunization - Groups of N=10 BALB/c

mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-IApol

(E3+) at either 10^7 vp and 10^9 vp; and (2) MRKAd5hCMV-IApol (E3-) at either

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10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively. For all rodent immunizations, the Ad5 vectors were diluted in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl2, 0.005% polysorbate 80, pH 8.0. The total dose was injected to both quadricep muscles in 50 µL aliquots using a 0.3-mL insulin syringe with 28-1/2G needles (Becton-Dickinson, Franklin Lakes, NJ).

Groups of N=10 C57/BL6 mice were immunized i.m. with the following vectors: (1) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; (2) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and 10^9 vp; and (3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. At 7 weeks post dose, 5 of the 10 mice per cohort were boosted with the same vector and dose they initially received. At 3 weeks post the second does, sera and spleens were collected from all the animals for RT ELISA and IFNg ELIspot analyses, respectively.

Non-human Primate immunization - Cohorts of 3 rhesus macaques (2-3 kg) were vaccinated with the following Ad vectors: (1) MRKAd5hCMV-IApol (E3+) at either 10^9 vp and 10^11 vp dose; and (2) MRKAd5hCMV-IApol (E3-) at either 10^9 vp and 10^11 vp; (3) MRKAd5hCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp; and (4) MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^9 vp and 10^11 vp. The vaccine was administered to chemically restrained monkeys (10 mg/kg ketamine) by needle injection of two 0.5 mL aliquots of the Ad vectors (in 5 mM Tris, 5% sucrose, 75 mM NaCl, 1 mM MgCl₂, 0.005% polysorbate 80, pH 8.0) into both deltoid muscles. The animals were immunized twice at a 4 week interval (T=0, 4 weeks).

Murine anti-RT and anti-nef ELISA - Anti-RT titers were obtained following standard secondary antibody-based ELISA. Maxisorp plates (NUNC, Rochester; NY) were coated by overnight incubation with 100 μL of 1 μg/mL HIV-1 RT protein (Advanced Biotechnologies, Columbia, MD) in PBS. For anti-nef ELISA, 100 uL of 1 ug/mL HIV-1 nef (Advanced Biotechnologies, Columbia, MD) was used to coat the plates. The plates were washed with PBS/0.05% Tween 20 using Titertek MAP instrument (Hunstville, AL) and incubated for 2 h with 200 μL/well of blocking solution (PBS/0.05% tween/1% BSA). An initial serum dilution of 100-fold was performed followed by 4-fold serial dilution. 100-μL aliquots of serially diluted samples were added per well and incubated for 2 h at room temperature. The plates

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were washed and 100 μ L of 1/1000-diluted HRP-rabbit anti-mouse IgG (ZYMED, San Francisco, CA) were added with 1 h incubation. The plates were washed thoroughly and soaked with 100 μ L 1,2-phenylenediamine dihydrochloride/hydrogen peroxide (DAKO, Norway) solution for 15 min. The reaction was quenched by adding 100 μ L of 0.5M H₂SO4 per well. OD₄₉₂ readings were recorded using Titertek Multiskan MCC/340 with S20 stacker. Endpoint titers were defined as the highest serum dilution that resulted in an absorbance value of greater than or equal to 0.1 OD₄₉₂ (2.5 times the background value).

Non-human primate and murine ELIspot assays - The enzyme-linked immuno-spot (ELISpot) assay was utilized to enumerate antigen-specific INFy-10 secreting cells from mouse spleens (Miyahira, et al.1995, J. Immunol. Methods 181:45-54) or macaque PBMCs. Mouse spleens were pooled from 5 mice/cohort and single cell suspensions were prepared at 5x106/mL in complete RPMI media (RPMI1640, 10% FBS, 2mM L-glutamine, 100U/mL Penicillin, 100 u/mL streptomycin, 10 mM Hepes, 50 uM β -ME). Rhesus PBMCs were prepared from 8-15 15 mL of heparinized blood following standard Ficoll gradient separation (Coligan, et al, 1998, Current Protocols in Immunology. John Wiley & Sons, Inc.). Multiscreen opaque plates (Millipore, France) were coated with 100 µL/well of either 5 µg/mL purified rat anti-mouse IFN-y IgG1, clone R4-6A2 (Pharmingen, San Diego, CA), or 15 ug/mL mouse anti-human IFN-γ IgG_{2a} (Cat. No. 1598-00, R&D Systems, 20 Minneapolis, MN) in PBS at 4°C overnight for murine or monkey assays, respectively. The plates were washed with PBS/penicillin/streptomycin and blocked with 200 µL/well of complete RPMI media for 37 °C for at least 2 h.

To each well, 50 μL of cell samples (4-5x10⁵ cells per well) and 50 μL of the antigen solution were added. To the control well, 50 μL of the media containing DMSO were added; for specific responses, either selected peptides or peptide pools (4 ug/mL per peptide final concentration) were added. For BALB/c mice immunized with the pol constructs, stimulation was conducted using a pool of CD4⁺-epitope containing 20-mer peptides (aa21-40, aa411-430, aa641-660, aa731-750, aa771-790) or a pool of CD8⁺-epitope containing peptides (aa201-220, aa311-330, aa781-800). For C57/BL6 mice immunized with the nef construct, either aa51-70 (CD8⁺T cell epitope) or aa81-100 (CD4⁺) peptide derived from the nef sequence was added for specific stimulation. In monkeys, the responses against pol were evaluated using two pools (L and R) of 20-aa peptides that encompass the entire pol sequence and overlap by 10 amino acids. In monkeys vaccinated with the nef constructs, a single pool containing 20-mer peptides covering the entire HTV-1 nef sequence and overlapping

by 10 aa was used. Each sample/antigen mixture was performed in triplicate wells for murine samples or in duplicate wells for rhesus PBMCs. Plates were incubated at 37°C, 5% CO₂, 90% humidity for 20-24 h. The plates were washed with PBS/0.05% Tween 20 and incubated with 100 μL/well of either 1.25 μg/mL biotin-conjugated rat anti-mouse IFN-γ mAb, clone XMG1.2 (Pharmingen) or of 0.1 ug/mL biotinylated anti-human IFN-gamma goat polyclonal antibody (R&D Systems) at 4°C overnight. The plates were washed and incubated with 100 μL/well 1/2500 dilution of strepavidin-alkaline phosphatase conjugate (Pharmingen) in PBS/0.005% Tween/5% FBS for 30 min at 37 °C. Spots were developed by incubating with 100 μL/well 1-step NBT/BCIP (Pierce Chemicals) for 6-10 min. The plates were washed with water and allowed to air dry. The number of spots in each well was determined using a dissecting microscope and the data normalized to 10⁶ cell input.

Non-human Primate anti-RT ELISA - The pol-specific antibodies in the monkeys were measured in a competitive RT EIA assay, wherein sample activity is determined by the ability to block RT antigen from binding to coating antibody on the plate well. Briefly, Maxisorp plates were coated with saturating amounts of pol positive human serum (#97111234). 250 uL of each sample is incubated with 15 uL of 266 ng/mL RT recombinant protein (in RCM 563, 1% BSA, 0.1% tween, 0.1% NaN₃) and 20 uL of lysis buffer (Coulter p24 antigen assay kit) for 15 min at room temperature. Similar mixtures are prepared using serially diluted samples of a standard and a negative control which defines maximum RT binding. 200 uL/well of each sample and standard were added to the washed plate and the plate incubated 16-24 h at room temperature. Bound RT is quantified following the procedures described in Coulter p24 assay kit and reported in milliMerck units per mL arbitrarily defined by the chosen standard.

Results - Rodent Studies - BALB/c mice (n=5 mice/cohort) were immunized once or twice with varying doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). At 3 weeks after the second dose, Anti-pol IgG levels were determined by an ELISA assay using RT as a surrogate antigen. Cellular response were quantified via IFNY ELISpot assay against pools of pol-epitope containing peptides. The results of these assays are summarized in Table 10. The results indicate that the mouse vaccinees exhibited detectable anti-RT IgGs with an adenovector dose as low as 10^7 vp. The humoral responses are highly dose-dependent and are boostable with a second immunization. One or two doses of either pol vectors elicit high frequencies of antigen-specific CD4⁺ and CD8⁺ T cells; the responses are weakly dose-dependent but are boostable with a second immunization.

Table 10. Immunogenicity of MRKAd5pol Vectors in BALB/c mice.

	TO. MANAGEMENT											
				Anti-RT IgG Titers*								<u>s•</u>
Group	Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medium	CD4+ peptide pool	CD8+ peptide pool			
1	MRKAd5hCMVFLpol (E3+)	10^7 vp	2 1	310419 919	301785 372	153020 265	1(1) 1(1)	75(4) 72(9)	2313(87) 533(41)			
2	MRKAd5hCMVFLpol (E3+)	10^9 vp	2	1638400 ⁶ 713155	0 528520	0 303555	2(2) 1(1)	114(9) 48(7)	2063(182) 733(89)			
3	MRKAd5hCMVFLpol (E3-)	10^7 vp	2	310419 6400	386218 14013	172097 4393	0(0) 10(8)	223(7) 141(21)	2607(27) 409(28)			
4	MRKAd5hCMVFLpol (E3-)	10^9 vp	2	1838400 ^b 1241675 ^b	0 396725	0 300661	1(1) 0(0)	160(13) 39(13)	2385(11) 833(83)			
5	Naïve	none	none	57	9	7	9(2)	11(4)	10(1)			

^{*}GMT, geometric mean titer of the cohort of 5 mice; SE, standard error of the gemetric mean

C57/BL6 mice were immunized once or twice with varying doses of MRKAd5hCMV-nef(G2A,LLAA) (E3+), MRKAd5mCMV-nef(G2A,LLAA) (E3+) at either 10^7 vp and(3) MRKAd5mCMV-tpanef(LLAA) (E3+) at either 10^7 vp and 10^9 vp. The immune response were analyzed using similar protocols and the results are listed in Table 11. While anti-nef IgG responses could not be detected in this model system with any of the constructs, there are strong indications of a cellular immunity generated against nef using the ELIspot assay.

Table 11. Immunogenicity of MRKAd5nef Vectors in C57/BL6 mice.

			Ап	ti-nef IgG Tite	18	SFC/10^6 cells				
Group Vaccine	Dose	No. of Doses	GMT	+SE	-SE	Medium	aa51-70 CD8+	881-100 CD4+		
1	MRKAd5hCMVFLnet (E3+)	10^7 vp	2	174	70	50	1(1)	23(1)	1(1)	
•	,,		1	132	42	32	0(0)	0(0)	0(0)	
-2-	MRKAd5hCMVFLnef (E3+)	10^9 vp	2	174	70	50	0(0)	61(7)	4(2)	
	•		1 1	132	42	32	1(1)	62(7)	3(1)	
-3-	MRKAd5mCMVFLnef (E3+)	10^7 vp	2	132	42	32	3(1)	15(5)	5(2)	
) i	1 -	115	48	33	3(2)	3(2)	4(2)	
4	MRKAd5mCMVFLnef (E3+)	10^9 vp	2	132	42	32	4(2)	83(13)	5(1)	
-			1	132	42	32	2(1)	29(2)	4(0)	
5	MRKAd5mCMVtpanef(E3+)	10^7 vp	2	132	42	32	3(2)	14(2)	5(1)	
	, ,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		1	100	0	0	3(1)	13(4)	10(3)	
6	MRKAd5mCMVtpanef(E3+)	10/9 vp	2	230	170	98	3(2)	145(29)	4(0)	
_			1	115	46	33	7(1)	151(14)	10(0)	
7	Naïve	none	none	152	78	52 -	21(2)	· 18(6)	28(3)	

^{*}GMT, geometric mean titler of the cohort of 5 mice; SE, standard error of the gemetric mean

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Monkey Studies - Cohorts of 3 rhesus macaques were immunized with 2 doses of MRKAd5hCMV-IApol(E3+) and MRKAd5hCMV-IApol(E3-). The number of antigen-specific T cells (per million PBMCs) were enumerated using one of two

Near or at the upper limit of the serial dilution; hence, could be greater than this value

No. of Spot-forming Cells per million splechoytes; mean values of triplicates are reported along with standard errors in parenthesis.

No. of spot-forming cells per million splecnoyles; mean values of triplicates are reported along with standard errors in parenthesis.

peptide pools (L and R) that cover the entire pol sequence; the results are listed in Table 12. Moderate-to-strong T cell responses were detected in the vaccinees using either constructs even at a low dose of 10^9 vp. Longitudinal analyses of the anti-RT antibody titers in the animals suggest that the pol transgene product is expressed efficiently to elicit a humoral response (Table 13). It would appear that generally higher immune responses were observed in animals that received the E3- construct compared to the E3+ virus.

Table 12. Pol-specific T Cell Responses in MRKAd5pol Immunized Rhesus

Macaques.

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Vaccine (T=0,4 wks)	Monk #		Prebleed			T=4		_	T=7		L	T=16	
		Mock	Pol L	Pol R	Mock	Pol L	Pol R	Mock	PolL	Pol R	Mock	Pol L	Pol R
MRKAd5hCMV-IApot(E3+)	990100	1	0	0	1	38	31	0	52	146	0	49	715
10^11 vp	99C215	1	2	2	10	98	249	1	109	305	22	88	250
id ii VP	99D201	5	5	4	6	149	95	0	40	35	0	35	18
MRKAd5hCMV-IApol(E3+)	99D212	0	2	0	4	331	114	0	58	14	0	6	6
10/9 VD	99D180	0	4	2	0	19	192	4	36	158	- 5	38	108
•	99C201	8	5	21	6	62	82	٥	18	32	} '	14	65
MRKAdShCMV-IApol(E3-)	990239	5	2	2	20	82	172	1	68	114	9	21	40
1041 vp	99C186	4	12	6	5	120	421	2	271	489	16	875	530
·	99C084	1	8	9	8	84	464	0	14	236	1	24	264
MRKAdShCMV-IApol(E3-)	CC7C	10	10	8	12	724	745	4	322	376	4	188	176
10/9 VD	ထၢေ	2	0	1	5	474	468	0	232	212	0	101	121
	CD11	6	6	12	10	98	110	5	60	80	8	25	34
Nave	083Q	nd	nd	nd	nd	nd	nd	4	2	2	2	1	2

nd, not determined Reported are SFC per million PBMCs; mean of duplicate wells.

Table 13. Anti-RT Ig Levels in MRKAd5pol Immunized macaques.

RT ANTIBODY ASSAY TITERS IN MMU	mL			
Vaccine/Monkey Tag	T=4	T =7	T=12	T=16
MRKAd5hCMV-IApol(E3+), 10^11 vp				
99C100	61	1999	5928	4768
99C215	81	1541	2356	2767
99D201	53	336	539	387
MRKAd5hCMV-IApol(E3+), 10^9 vp			· · · · · · · · · · · · · · · · · · ·	
99D212	10	40	49	68
99D180	<10	36	79	93
99C201	<10	37	71	76
MRKAd5hCMV-IApol(E3-), 10^11 vp				
99D239	44	460	1234	1015
99C186	21	· 233 ·	480	345
990084	235	2637	2858	1626
MRKAd5hCMV-IApol(E3-), 10^9 vp	-			
CC7C	32	175	306	235
Ø16	20	140	273	419
Q11	15	112	149	237

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When rhesus macaques were immunized i.m. with two doses of MRKAd5nef constructs, vigorous T cell responses ranging from 100 to as high as 1100 per million were observed in 8 of 12 vaccinees (Table 14). The efficacies of the mCMV- and hCMV- driven nef constructs are comparable on the basis of the data generated thus far.

Table 14. Nef-specific T cell Responses in MRKAd5nef Immunized Rhesus Macaques.

Vaccine (T=0,4 wks)	Monk#	Pi	re	T=4		T=7		T=16	
		Mock	Nef	Mock	Nef	Mock	Nef	Mock	Nef
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CD2D	0	4	31	440	4	368	1	251
10^11 vp	CC7B	0	0	2	521	0	178	1	1522
	CC61	2	9	31	112	0	108	11	100
MRKAd5hCMV-nef(G2A,LLAA) (E3+)	CC2K	9	9	6	52	0	35	0	15
10/9 vp	CD15	5	4	30	998	2	586	0	434
	CD16	6	1	6	1146	0	369	1	212
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D191	1	5	4	614	0	298	2	419
10^11 vp	99D144	4	6	5	434	0	1100	2	932
·	99C193	1 1	2	1	58	1	22	٥	64
MRKAd5mCMV-nef(G2A,LLAA) (E3+)	99D224	1	11	14	231	1	125	0	70
10^9 vp	99D250	8	9	4	108	0	54	0	5
•	99C120	1	6	20	299	0	92	0	79
Naîve	083Q	nd	nd	18	22	4	5	2	1

EXAMPLE 25

Comparison of Clade B vs. Clade C T Cell Responses in HIV-Infected Subjects

PBMC samples collected from two dozens of patients infected with HIV-1 in

US were tested in ELISPOT assays with peptide pools of 20-mer peptides overlapping
by 10 amino acids. Four different peptide pools were tested for cross-clade
recognition, and they were either derived from a clade B-based isolate (gag H-b; nefb) or a clade C-based isolate (gag H-c, nef-c). Data in Table 15 shows that T cells
from these patients presumably infected with clade B HIV-1 could recognize clade C
gag and nef antigens in ELISPOT assay. Correlation analysis further demonstrated
that these T cell responses against clade C gag peptide pool were about 60% of the
clade B counterpart (Figure 24), while the T cell responses against clade C nef were
about 85% of the clade B counterpart (Figure 25). These results suggest that cellular
immune responses generated in patients infected with clade B HIV-1 can recognize
gag and nef antigens derived from clade C HIV-1. These data show that a HIV
vaccine, such as a DNA or MRKAd5-based adenoviral vaccine expressing a clade B

gag and/or nef antigen will potentially have the ability to provide a prophylactic and/or therapetic advantage on a global scale.

Table 15
Responses Shown as the Number of gIFN-Secreting T Cells per Million PBMCs

subject	bleed date	gag epitope #	mock	gag H-b	gagH-c	nef-b	nef-c
		from mapping)					
#100	19-Jul-99	12	10	3950	1385	1295	1300
#101	25-Jul-99	3	15	3885	1280	na	1020
#102	25-Jul-99	4	15	1740	850	1255	1785
#104	7-Jun-99	2	5	1355	1185	na	1060
#107	11-Oct-99	2	25	3305	2795	670	870
#405	11-Jul-99	2	15	4575	3180	1700	1500
#501	19-Jul-99	2	15	1100	570	3365	3460
#505	18-Jul-99	5	10	2145	1725	1235	na
#506	28-Feb-99	2	25	150	45	400	610
#701	28-Mar-99	5	30	7620	4775	3320	2780
#709	17-May-99	3	15	2785	1945	1090	1630
#710	24-May-99	4	5	1055	1080	2210	2140

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EXAMPLE 26

Characterization and Production of MRKAd5pol and MRKAd5nef Vectors in Roller Bottles

Expansion of nef and pol Adenovectors - Nef and pol CsCl purified MRKAd5 seeds were used to infect roller bottles to produce P4 virus to be used as a seed for further experiments. P4 MRKAd5 pol and nef vectors were used to infect roller bottles at an MOI 280 vp/cell, except for hCMV-tpa-nef [E3+] which was infected at an MOI of 125 due to low titers of seed obtained at P4.

Table 16 Viral particle concentrations for P5 nef and pol adenovectors

Adenovector	AEX Titer (10 ¹⁰ vp/ml culture)	AEX Titer (10 ⁴ vp/cell)	Amplification Ratio
hCMV-FL-nef [E3+]	1.1	0.9	30
mCMV-FL-nef [E3+]	2.2	2.1	75
hCMV-tpa-nef [E3+]	0.07	0.1	5
mCMV-tpa-nef [E3+]	1.3	0.9	35
hCMV-FL-pol [E3+]	2.7	2.1	75
hCMV-FL-pol [E3-]	1.9	1.3	45

Roller Bottle Passaging - Passaging of the pol and nef constructs continued through passage seven. Cell-associated (freeze/thaw lysis) and whole broth (tritonlysis) titers obtained in all passages were very consistent. In general, MRKAd5pol is ca. 70% as productive as MRKAd5gag while MRKAd5nef is ca. 25% as productive as MRKAd5gag. Samples of P7 virus for both constructs were analyzed by V&CB by restriction digest analysis and did not show any rearrangements.

Table 17. Passage Six Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10 ⁴ cells/ml), Viability (%) Infection Harvest		Cell Passage Number	AEX Titer (Cell Associated) 10 ¹⁰ vp/ml culture	Titer 104 vp/cell	Amplification Ratio	Triton Lysis Titer 10 ¹⁰ vp/ml culture
hCMV-FL-nef [B3+]	pool	1.22, 85%	<u> </u>	62	0.8	0.7	25	1.6
	1 2		0.99, 62% 1.10, 72%					
hCMV-FL-pol (E3+)	pool	1.42, 89%		62	4.5	3.2	115	7.0
	. 1		1.22, 70% 1.42, 74%					

15 Table 18. Passage Seven Viral Productivity for MRKAd5pol and MRKAd5nef

		Xviable (10 Viabili		Cell Passage	AEX Titer (Cell Associated)	Titer	Amplification	Triton Lysis Titer
	1	Infection	Harvest	Number	10 to vp/ml culture	10 ⁴ vp/ceII	Ratio	10 ¹⁰ vp/ml culture
bCMV-FL-ncf [E3+]	Pool	1.33, 90%		66	1.0	0.8	29	2.1
	1		0.96, 70%					
	2		1.18, 73%					
hCMV-FL-pol [E3+]	Pool	0.90*, 90%		56	4.2	4.7	168	6.5
	1		1.18, 88%					
	2		1.04, 80%	<u> </u>				

MRKAd5nef and MRKAd5pol Viral Production Kinetics - A timecourse experiment was carried out in roller bottles to determine if the viral production kinetics of the MRKAd5pol and MRKAd5nef vectors were similar to those of MRKAd5gag. PER.C6® cells in roller bottle cultures were infected at an MOI of 280 vp/cells with P5 MRKAd5pol, P5 MRKAd5nef and P7 MRKAd5gag; for each adenovector, two infected bottles were sampled at 24, 36, 48, and 60 hours post infection. In addition, two bottles were left unsampled until 48 hpi when they were harvested under the Phase I process conditions. The anion-exchange HPLC viral particle concentrations of the freeze-thaw recovered cell associated virus at the 24, 36,

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48, and 60 hpi timepoints are shown in Figure 29A-B. The QPA titers show a similar trend (data not shown).

Comparison of hCMV- and mCMV-FL-nef - As the titers obtained with the MRKAd5nef construct (hCMV-FL-nef) were lower than those obtained with MRKAd5gag or MRKAd5pol, a viral productivity comparison experiment was performed with mCMV-FL-nef. For each of the two adenovectors (hCMV- and mCMV-FL-nef), two roller bottles were infected at an MOI of 280 vp/cell with passage five clarified lysate. The macroscopic and microscopic observations of the four roller bottles were identical at the time of harvest. Analysis of the clarified lysate 10 produced indicated a higher viral particle concentration in the bottles infected with mCMV-FL-nef, as shown in Table 19. It is stipulated that the higher productivity with mCMV promoter driven nef vector is due to lower nef expression levels in PER.C6® cells- experiments are underway at V&CB to measure nef expression levels.

Table 19. Passage Six Viral Productivity Comparison of hCMV- and mCMV-FL-nef

		,	Xv (10 ⁶ cells/ml), Viability (%)		AEX Titer	Titer	Amplification	Triton Lysis Titer
		Infection	Harvest	Number	10 ¹⁰ vp/ml culture	. 10° vp/cell	Ratio	10 ¹⁰ vp/ml culture
hCMV-FL-nef	Pool	1.11, 91%		60	1.5	1.4	50	2.8
(MRKAd5nef)	. 1		1.23,75%					
	2		1.34,74%		1			
mCMV-FL-nef	Pool	1.11, 91%		60	2.3	2.1	75	4.6
	1		1.49, 84%					
	2		1.18,77%	1				

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EXAMPLE 27

Characterization and Large Scale Production of MRKAd5nef Virus in Bioreactors

Materials and Methods - The experiment of the present example was run twice under the following conditions: 36.5°C, DO 30%, pH 7.30, 150rpm agitation rate, no sparging, Life Technologies (Gibco, Invitrogen) 293 SFM II (with 6mM Lglutamine), 0.5M NaOH as base for pH control. During the first run (B20010115), two 10L stirred vessel bioreactors were inoculated with PER.C6® cells at a concentration of 0.2x10⁶ cells/ml. Cells were grown until they reached a cell concentration of approximately 1x106 cells/ml. The cells were infected with uncloned MRKAd5nef (G2A,LLAA) at a MOI of 280 virus particles (vp)/cell. For the second batch (B20010202), the same procedure as the first run was used, except the cells

were infected with cloned MRAd5nef. During both runs, the bioreactors were harvested 48 hours post-infection. Samples were taken and virus concentrations were determined from whole broth (with triton lysis), supernatant, and cell pellets (3 X freeze/thaw) with the AEX and QPA assays. Metabolites were measured with BioProfile 250 throughout the process.

Table 20: Experimental Conditions

Temperature	36.5 ℃
DO	30%
PH	7.30
Agitation	150 rpm
Sparging	None

Table 21: Virus source used for experiments.

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Run	Batch ID	Cloned/Uncloned	MOI
		MRKAd5nef	(vp/cells)
#1	B20010115-1	Uncloned	280
	B20010115-2	Uncloned	280
#2	B20010202-1	Cloned	280
	B20010202-2	Cloned	280

Results - Table 22 and 23 show an the ability to scale up production of MRKAd5nef by growth in a bioreactor.

Table 22: Virus Concentration as measured by the AEX assay

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Run	Batch ID	Cloned/Uncloned	V	Virus Concentration @ 48hpi (1x10 ¹³ vp/L)						
		MRKAd5nef	Supernatant	Clarified Lysate	Total	Triton Lysate				
#1	B20010115-1	Uncloned	0.72	3.26	3.98	5.76				
	B20010115-2	Uncloned	0.38	1.67	2.05	2.46				
#2	B20010202-1	Cloned	0.80	6.00	6.80	8.88				
"-	B20010202-2	Cloned	0.50	6.00	6.50	8.47				

Table 23: Virus Titers as measured by the QPA assay

Run	Batch ID	Cloned/Uncloned	Virus Concentration @ 48hpi (1x10 ¹¹ IU/L)						
		MRKAd5nef	Whole Broth	Supernatant	Clarified Lysate	Total	Triton Lysate		
#1	B20010115-1	Uncloned	0.13	1.12	1.76	2.88	11.28		
"-	B20010115-2	Uncloned	0.14	0.73	1.54	2.27	5.86		
#2	B20010202-1	Cloned	0.14	0.97	1.62	2.69	11.89		
"-	B20010202-2	Cloned	0.14	1.17	1.70	2.97	12.47		

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The present invention is not to be limited in scope by the specific embodiments described herein. Indeed, various modifications of the invention in addition to those described herein will become apparent to those skilled in the art

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from the foregoing description. Such modifications are intended to fall within the scope of the appended claims.

EXAMPLE 28

MRKAd5HIV-1gag Boosting of DNA-Primed Animals

Groups of 3-5 rhesus macaques were immunized with (a) 5 mgs of V1Jns-Flgag (pVIJnsCMV(no intron)-FL-gag-bGHpA), (b) 5 mgs of V1Jns-Flgag formulated with 45 mgs of a non-ionic block copolymer CRL1005, or (c) 5 mgs of V1Jns-Flgag formulated with 7.5 mgs of CRL1005 and 0.6 mM benzalkonium chloride at weeks 0, 4, and 8. All animals received a single dose of 10e7 viral particles (vp) of the MRKAd5HIV-1gag at week 26. Note: 10e7 is too low to prime or boost effectively when used as a single modality (dose is selected to mimic preexposure to adenovirus); see Figure 32.

Blood samples were collected from all animals at several time points and peripheral blood mononuclear cells (PBMCs) were prepared using standard Ficoll method. The PBMCs were counted and analyzed for gamma-interferon secretion using the ELISpot assay (Table 24). For each monkey, the PBMCs were incubated overnight either in the absence (medium) or presence of a pool (called "gag H") of 50 20-aa long peptides that encompass the entire HIV-1 gag sequence.

The results indicate that MRKAd5HIV-1gag was very effective in boosting the T cell immune responses in these monkeys. At week 28 or 2 weeks after the viral boost, the number of gag-specific T cells per million PBMCs increased 2-48 fold compared to the levels observed at week 24 or 2 weeks prior to the boost.

The PBMCs were also analyzed by intracellular gamma-interferon staining prior to (at week 10) and after the MRKAd5gag boost (at week 30). The results for select animals are shown on Figure 31. The results indicate that (a) immunization with DNA/adjuvant formulation elicited T cell responses which can either be balanced, CD4⁺-biased or CD8⁺-biased, and (b) boosting with the MRKAd5gag construct produced in all cases a strongly CD8⁺-biased response. These results suggest that boosting with MRKAd5HIV-1gag construct is able to improve the levels of antigen-specific CD8⁺ T cells.

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EXAMPLE 29

Construction of gagpol fusion for MRKAd5gagpol fusion constructs

The open reading frames for the codon-optimized HIV-1 gag gene was fused directly to the open reading frame of the IA pol gene (consisting of RT, RNAseH and integrase domains) by stepwise PCR. Because the gene (SEQ ID NO: 38) does not include the protease gene and the frameshift sequence, it encodes a single polypeptide of the combined size of p55, RT, RNAse H and integrase (1350 amino acids; SEQ ID NO: 39).

The fragment that extends from the BstEII site within the gag gene to the last non-stop codon was ligated via PCR to a fragment that extends from the start codon of the IApol to a unique BamHI site. This fragment was digested with BstEII and BamHI. Construction of gag-IApol fusion was achieved via three-fragment ligation involving the PstI-BstEII gag digestion fragment, the BstEII/BamHI digested PCR product and long PstI/BamHI V1R-FLpol backbone fragment.

The MRKAd5-gagpol adenovirus vector was constructed using the BglII fragment of the V1R-gagpol containing the entire ORF of gag-IApol fusion gene.

EXAMPLE 30

Immunogenicity Studies in Non-Human Primates

Cohorts of three (3) macaques were immunized with 10e8 or 10e10 viral particles (vp) of one of the following MRKAd5 HIV-1 vaccines: (1) MRKAd5gag; (2) MRKAd5pol; (3) MRKAd5nef; (4) a mixture containing equal amounts of MRKAd5gag, MRKAd5pol, and MRKAd5nef, or (5) a mixture of equal amounts of MRKAd5gagpol and MRKAd5nef. The vaccines were administered at weeks 0 and 4.

The T cell responses against each of the HIV-1 antigens were assayed by IFN-gamma ELISpot assay using pools of 20-aa peptides that encompass the entire protein sequence of each antigen. The results (Table 25) are expressed as the number of spot-forming cells (sfc) per million peripheral blood mononuclear cells (PBMC) that respond to each of the peptide pools.

Results indicate the following observations: (1) each of the single gene constructs (MRKAd5gag, MRKAd5pol, or MRKAd5nef) is able to elicit high levels of antigen-specific T cells in monkeys; (2) the single-gene MRKAd5 constructs can be mixed as a multi-cocktail formulation capable of eliciting very broad T cell responses against gag, pol, and nef; (3) the MRKAd5 vector expressing the fusion

protein of gag plus IA pol is capable of inducing strong T cell responses to both gag and pol.

Table 25. Evaluation of Mixtures of MRKAd5 vectors expressing humanized

5 HIV-1 gag, pol, gagpol, nef in rhesus macaques

Grp#	Vaccine	Monk#							
•	T=0, 4 wks		Mock	Gag H	Pol - 1	Pol-2	Nef		
1	MRKAd5 gag	CB9V	0	15	-	-	-		
	10^10 vp	CD19	0 .	374	-	-	-		
		109H	1	843	-	-	-		
2	MRKAd5 gag	99D130	1	948	•	•	•		
	10^8 vp	W277	16	324		•	-		
		143H	4	595	-	•	•		
3	MRKAd5 pol	CC1X	4	-	46	256	-		
·	10^10 vp	WEWA	3	-	463	550	-		
	•	AV43	6	-	95	1333	-		
4	MRKAd5 pol	AW38	1		19	30	-		
	10^8 vp	CC8K	0	-	50	995	-		
		CC21	1	-	33	436	-		
5	MRKAd5 nef	076Q	9	-	-	-	1204		
l	10^10 vp	091Q	4		-		85		
1		083Q	0	-	-	•	176		
6	MRKAd5 nef	00C029	1	-	-	-	114		
	10^8 vp	98D022	6	•	-	-	170		
1		98D160	3	•	-	-	198		
7	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D251	3	206	15	193	120		
	10^10 vp each	05H	3	135	21	9	638		
		00C016	3	26	4	51	23		
8	MRKAd5gag+MRKAd5pol+MRKAd5nef	99D215	1	171	18	193	240		
	10^8 vp each	81H	5	73	6	14	243		
	·	12H	8	1140	115	811	719		
9	MRKAd5gagpol +MRKAd5 nef	99D211	0	83	56	838	725		
	10^10 vp each	22H	4	385	119	1194	1915		
		61H	4	343	11	765	853		
10	MRKAd5gagpol +MRKAd5 nef	34H	3	78	19	5	75		
	10^8 vp each	48H	1 1	65	105	46	43		
		70H	5	158	15	220	191		

Indicated are numbers of spot-forming cells per million PBMCS against the peptide pools. Mock, no peptides; gag H, fifty 20-aa peptides encompassing p55 sequence; pol-1, 20-aa peptides representing N-terminal half of IA pol; pol-2, 20-aa peptides representing the carboxy-terminal half of IA pol; nef, 20-aa peptides encompassing the entire wild-type nef sequence. Responses to the antigens prior to the first immunization did not exceed 40 sfc/10⁶ PBMC.

WHAT IS CLAIMED IS

- A recombinant adenoviral vaccine vector at least partially deleted in
 E1 and devoid of E1 activity, comprising:
 - a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to between from about base pair 400 to about base pair 458 of a wildtype adenovirus genome; and
 - b) a gene encoding an HIV protein or immunologically relevant modification thereof.
 - 2. A vector in accordance with claim 1 comprising a packaging region corresponding to from about base pair 1 to about base pair 450 of a wildtype adenovirus genome.
- 3. A vector in accordance with claim 1 further comprising nucleotides
 15 corresponding to between from about base pair 3511 to about 3524 to about base pair
 5798 of a wildtype adenovirus genome.
 - 4. A vector in accordance with claim 3 comprising base pairs corresponding to 1-450 and 3511-5798 of a wildtype adenovirus genome.
- 5. A vector in accordance with claim 4 which is deleted of base pairs451-3510.
 - 6. A vector in accordance with claim 1 which is at least partially deleted in E3.
 - 7. A vector in accordance with claim 6 wherein the E3 deleted region is from base pairs 28,133-30,818.

- 8. A vector in accordance with claim 1 wherein the gene encoding the HIV protein or modification thereof comprises codons optimized for expression in a human.
- 9. A vector in accordance with claim 1 wherein the vector comprises a5 gene expression cassette comprising:
 - a) a nucleic acid encoding a protein;
 - b) a heterologous promoter operatively linked to the nucleic acid encoding the protein; and
 - (c) a transcription termination sequence.
- 10. A vector in accordance with claim 9 wherein the gene expression cassette is inserted into the E1 region.
 - 11. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette is in an E1 parallel orientation
- 12. An adenoviral vector in accordance with claim 9 wherein the geneexpression cassette is in an E1 antiparallel orientation.
 - 13. An adenoviral vector in accordance with claim 9 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
 - 14. An adenoviral vector in accordance with claim 13 wherein the promoter is an immediate early human cytomegalovirus promoter.
- 20 15. An adenoviral vector in accordance with claim 9 wherein the promoter is a murine cytomegalovirus promoter.
 - 16. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.

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17. An adenoviral vector in accordance with claim 9 wherein the transcription termination sequence is a synthetic polyadenylation signal (SPA).

- 18. A cell comprising the adenoviral vector of claim 1.
- 19. Recombinant, replication-defective adenovirus particles harvested
 and purified subsequent to transfection of the adenoviral vector of claim 1 into a cell
 line which expresses adenovirus E1 protein at complementing levels.
 - 20. An HIV vaccine composition comprising purified adenovirus particles of claim 19.
- 21. An HIV vaccine composition of claim 20 which comprises a physiologically acceptable carrier.
 - 22. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 1 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 23. A method according to claim 22 wherein the cell is a PER.C6® cell.

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- 24. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.
- 25. A method according to claim 24 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.

- 26. A method according to claim 25 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
- 27. A method according to claim 24 wherein the adenovirus vaccine is
 5 preceded by an adenovirus vaccine of a different serotype.
 - 28. A method according to claim 24 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 29. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV gag or an immunologically relevant modification thereof.
- 30. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV gag protein or immunologically relevant modification thereof.
 - 31. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
- a) an adenovirus cis-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - i) SEQ ID NO: 29;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.

- 32. An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 parallel orientation.
- 33 An adenoviral vector in accordance with claim 31 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 34. An adenoviral vector in accordance with claim 31 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 35. An adenoviral vector in accordance with claim 31 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 36. An adenoviral vector in accordance with claim 31 which is at least partially deleted in E3.
 - 37. A cell comprising the adenoviral vector of claim 30.
 - 38. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 30 into a cell line which expresses adenovirus E1 protein at complementing levels.
 - 39. An HIV vaccine composition comprising purified adenovirus particles of claim 38.
 - 40. An HIV vaccine composition of claim 39 which comprises a physiologically acceptable carrier.
- 41. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 30 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.

- 42. A method according to claim 41 wherein the cell is a PER.C6[®] cell.
- 43. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 21.
- 44. A method according to claim 43 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
- 45. A method according to claim 44 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
 - 46. A method according to claim 43 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 47. A method according to claim 43 which comprises administering and readministering the adenovirus vaccine vector to the individual.
 - 48. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV pol or an immunologically relevant modification thereof.
- 49. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV pol protein or immunologically relevant modification thereof.
 - 50. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:

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- b) a gene expression cassette comprising
 - i) a nucleotide sequence selected the group consisting of SEQ ID NO: 1, SEQ ID NO: 5 and SEQ ID NO: 7;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.
- 51. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 parallel orientation.
- 52. An adenoviral vector in accordance with claim 50 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 53. An adenoviral vector in accordance with claim 50 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
 - 54. An adenoviral vector in accordance with claim 50 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 55. An adenoviral vector in accordance with claim 50 which is at least partially deleted in E3.
 - 56. A cell comprising the adenoviral vector of claim 49.

- 57. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 49 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 58. An HIV vaccine composition comprising purified adenovirus particles of claim 57.
 - 59. An HIV vaccine composition of claim 58 which comprises a physiologically acceptable carrier.
 - 60. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 49 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 61. A method according to claim 60 wherein the cell is a PER.C6® cell.
- 62. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 59.
 - 63. A method according to claim 62 which further comprises administration to the individual a DNA plasmid vaccine, optionally administered with a biologically effective adjuvant, protein or other agent capable of increasing the immune response.
 - 64. A method according to claim 63 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.

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- 65. A method according to claim 62 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
- 66. A method according to claim 62 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- 5 67. An adenoviral vector in accordance with claim 1 wherein the HIV protein is HIV nef or an immunologically relevant modification thereof.
 - 68. An adenoviral vector in accordance with claim 9 wherein the gene expression cassette comprises an open reading frame encoding an HIV nef protein or immunologically relevant modification thereof.
 - 69. A recombinant adenoviral vaccine vector at least partially deleted in E1 and devoid of E1 activity, comprising:
 - a) an adenovirus *cis*-acting packaging region corresponding to from about base pair 1 to about base pair 450 and from about 3511 to about 5798 of a wildtype adenovirus genome, and deleted for base pairs corresponding to from about base pair 451 to from about base pair 3510 of a wildtype adenovirus genome; and
 - b) a gene expression cassette comprising
 - a nucleotide sequence selected the group consisting of SEQ ID NO: 9, SEQ ID NO: 11, SEQ ID NO: 13 and SEQ ID NO: 15;
 - ii) a heterologous promoter operatively linked to i); and
 - iii) a transcription termination sequence.
 - 70. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 parallel orientation.

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- 71. An adenoviral vector in accordance with claim 69 wherein the gene expression cassette is in an E1 antiparallel orientation.
- 72. An adenoviral vector in accordance with claim 69 wherein the promoter is a cytomegalovirus promoter devoid of intronic sequences.
- 73. An adenoviral vector in accordance with claim 69 wherein the transcription termination sequence is a bovine growth hormone polyadenylation and transcription termination sequence.
- 74. An adenoviral vector in accordance with claim 69 which is at least partially deleted in E3.
 - 75. A cell comprising the adenoviral vector of claim 68.
- 76. Recombinant, replication-defective adenovirus particles harvested and purified subsequent to transfection of the adenoviral vector of claim 68 into a cell line which expresses adenovirus E1 protein at complementing levels.
- 77. An HIV vaccine composition comprising purified adenovirus particles of claim 76.
 - 78. An HIV vaccine composition of claim 77 which comprises a physiologically acceptable carrier.
 - 79. A method of producing recombinant, replication defective adenovirus particles containing the adenoviral genome of the adenoviral vector of claim 68 which comprises introducing the adenoviral vector into a host cell which expresses adenoviral E1 protein, and harvesting the resultant recombinant, replication-defective adenovirus.
 - 80. A method according to claim 79 wherein the cell is a PER.C6® cell.

- 81. A method of generating a cellular-mediated immune response against HIV in an individual comprising administering to the individual a vaccine of claim 78.
- 82. A method according to claim 81 which further comprises

 5 administration to the individual a DNA plasmid vaccine, optionally administered with
 a biologically effective adjuvant, protein or other agent capable of increasing the
 immune response.
- 83. A method according to claim 82 wherein the DNA plasmid vaccine is administered to the individual prior to administration of an adenovirus vaccine.
 - 84. A method according to claim 81 wherein the adenovirus vaccine is preceded by an adenovirus vaccine of a different serotype.
 - 85. A method according to claim 81 which comprises administering and readministering the adenovirus vaccine vector to the individual.
- recombinant, replication-defective adenovirus particles, wherein the adenovirus particles are harvested and purified from a cell line expressing adenovirus E1 protein, and wherein the particles are harvested subsequent to transfection of the cells with an adenoviral vector or vectors in accordance with claim 9; said vector(s) comprising a gene expression cassette or cassettes comprising nucleotide sequences encoding HIV proteins selected from the group consisting of:
 - a) gag, pol, and nef, expressed independently from three individual vectors;

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b) gag, pol, and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct

promoters and transcription termination sequences;

- gag, pol, and nef, expressed via two vectors, one expressing a polnef fusion, and another expressing gag;
- d) gag, pol, and nef, expressed via two vectors, one expressing a gagpol fusion and another expressing nef;
- e) gag, pol and nef, expressed via two vectors, one expressing a nefgag fusion and another expressing pol;
- f) gag, pol, and nef, expressed via one vector expressing a gag-polnef fusion;
- g) gag and pol, expressed independently from two individual vectors;
- h) gag and pol, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- i) pol and nef, expressed independently from two individual vectors;
- j) pol and nef, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- k) nef and gag, expressed independently from two individual vectors;
- nef and gag, expressed independently from one vector with the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences;
- m) gag and pol, expressed via one vector expressing a gag-pol fusion;

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- n) pol and nef, expressed via one vector expressing a pol-nef fusion; and
- o) nef and gag, expressed via one vector expressing a nef-gag fusion.
- 87. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the gag-pol fusion consists of SEQ ID NO: 39.
 - 88. A multivalent adenovirus vaccine composition in accordance with claim 86 wherein the fused sequences have the encoding nucleic acid sequences operatively linked to distinct promoters and transcription termination sequences.
- 89. A multivalent adenovirus vaccine composition in accordance with

 10 claim 86 wherein the fused sequences have the encoding nucleic acid sequences

 operatively linked to a single promoter; and the encoding nucleic acid sequences

 operatively linked by an internal ribosome entry sequence ("IRES").

Original Adenovector Construct:

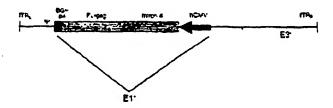


Figure 1: Original HIV-1 gag adenovector.

Sequence of the open reading frame for FL-gag (human codon optimized)

atgqqtqctagggcttctgtgctgtctggtggtgagctggacaagtgggagaagatcaggctgaggcctggtgg caaqaaqaaqtacaagctaaagcacattgtgtgggcctccagggagctggagaggtttgctgtgaaccctggc agetgaggteectgtacaacacagtggetaccetgtactgtgtgcaccagaagattgatgtgaaggacaccaag gaggecetggagaagattgaggaggagcagaacaagtecaagaagaaggcccagcaggctgctgctggc a cagginate cag cagging to caga actac cocatting the caga acctor and the canada can be caused as a canada canada caga acctor and the canada canada canada caga actac cagging to capacitate canada caga actac cagging to capacitate caga acctor and the caga actac cagging to capacitate caga actac cagging to capacitate cagging to capacitate caga actac cagging to capacitate caga actac cattering to capacitate caga actac cagging to capacitate capacitate capgccatctcccccggaccctgaatgcctgggtgaaggtggtggaggaagaccttctccctgaggtgatccc catgiticitigeeetgicigagggigeeaeeeeeaggaeetgaacaceatgetgaacacagiggggggeeate aggetgecatgeagatgetgaaggagaceateaatgaggaggetgetgagtgggaeaggetgeateetgtge acgetggccccattgcccccggccagatgagggagcccaggggctctgacattgctggcaccacctccaccct ccaggagcagattggciggaigaccaacaaccccccatccctgtgggggaaatctacaagaggtggatcat ccigggccigaacaagatig:gaggatgtactcccccacctccatcciggacatcaggcagggccccaaggag cccttcagggactatgtggacaggttctacaagaccctgagggctgagcaggcctcccaggaggtgaagaact ggatgacagagaccctgctggtgcagaatgccaaccctgactgcaagaccatcctgaaggccctgggccctg gctgaggccatgtcccaggtgaccaactccgccaccatcatgatgcagaggggcaacttcaggaaccagag gaagacagtgaagtgcttcaactgtggcaaggtgggccacattgccaagaactgtagggccccccaggaaga ggcaaaatctggccctcccacaagggcaggcctggcaacttcctccagtccaggcctgagcccacagcccct agetglaccccttggcctccttgaggtccttgtttggcaacgacccttctcccagtaaaataaagcccgggca gat (SEQ ID NO: 29)

Figure 2

Old Transgene:



New Transgenes:

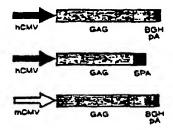


Figure 3: Diagrammatic representation of the original HIV-1 gag transgene and the series of new transgene constructions.

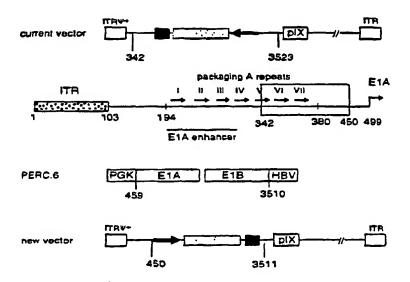


Figure 4: Modifications made to the current adenovector backbone in the generation of the new vector.

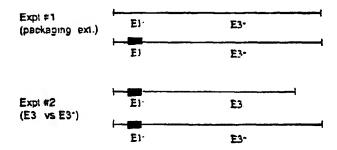


Figure 5: Virus mixing experiments to determine the effects of the addition made to the packaging signal region (Expt #1) and analysis of the effects of the E3 gene on viral growth (Expt. #2). The red bars denote the region of modifications made to the E1 deletion.



Figure 6: Autoradiograph of viral DNA analysis following viral mixing experiments (expts. #1 and #2) as detailed in the text.

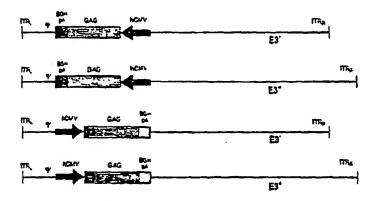


Figure 7A: hCMV-FLgag-bGHpA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

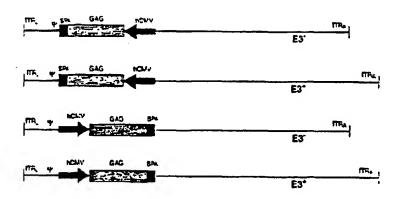


Figure 7B: hCMV-FLgag-SPA adenovectors constructed within the "MRK" backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

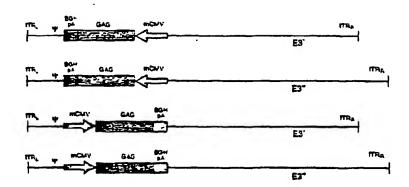


Figure 7C: mCMV-FLgag-bGHpA adenovectors constructed within the *MRK* backbone. E1 parallel and E1 antiparallel transgene orientation within the E3- and E3+ backbones were constructed.

Plasmid mixing expt: (orientation)

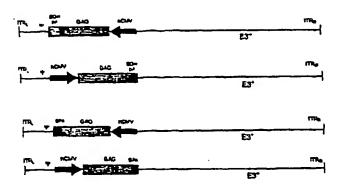


Figure 8A: Effect of transgene orientation

Plasmid Mixing expt: (poly A signal)

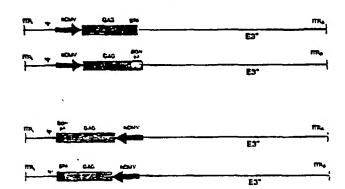


Figure 8B: Effect of polyadenylation signal



Figure 9: Viral DNA from the four Adgag candidates at P5, following BsfE11 digestion.

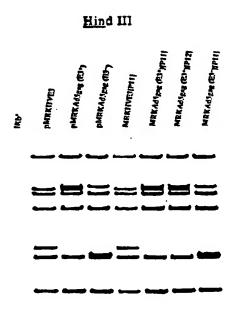


Figure 10: Viral DNA analysis of passage 11 and/or 12 of MRKHVE3, MRKAd5gag and MRKAd5gag(E3-).

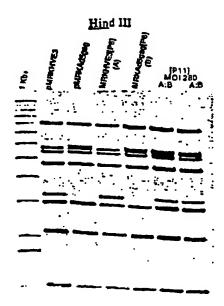


Figure 11: Viral DNA analysis (*Hin*dIII digestion) of passage 6 MRKHVE3 and MRKAd5gag used to initiate the viral competition study. Last two lanes are passage 11 analysis of duplicate passages of the competition study (each virus at MOI 280 vp).

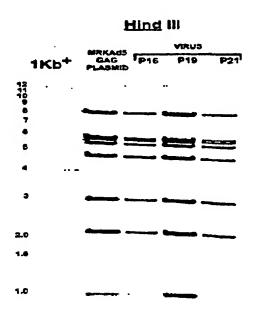
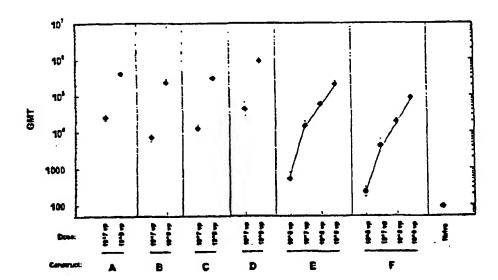


Figure 12: Viral DNA analysis by *Hin*dIII digestion on high passage numbers for MRKAd5gag in serum containing media with collections made at specified times. The first lane shows the 1 Kb DNA size marker. The other lanes represent pre-plasmid control (digested with Pac1 and *Hin*dIII), and MRKAd5gag virus continually passaged to P16, P19 and P21(serum containing media).

Figure . Serum anti-p24 Levels at 3 Wks post i.m. immunization of balb'c mice (n=10) with Varying Doses of Several Adgag constructs: (A) MRKAd5gag (through passage 5): (B) MRKAd5 E3 hCMV-FLgag-bGHpA; (C) MRKAd5 E3 hCMV-FLgag-SPA; (D) MRKAd5 E3 mCMV-FLgag-bGHpA; (D) research Lot (293 cell-derived) of Ad5HIV-1gag; and (F) clinical lot (Ad5gagFN0001) of Ad5HIV-1gag. Reponed are the geometric mean titers (GMT) for each cohort.



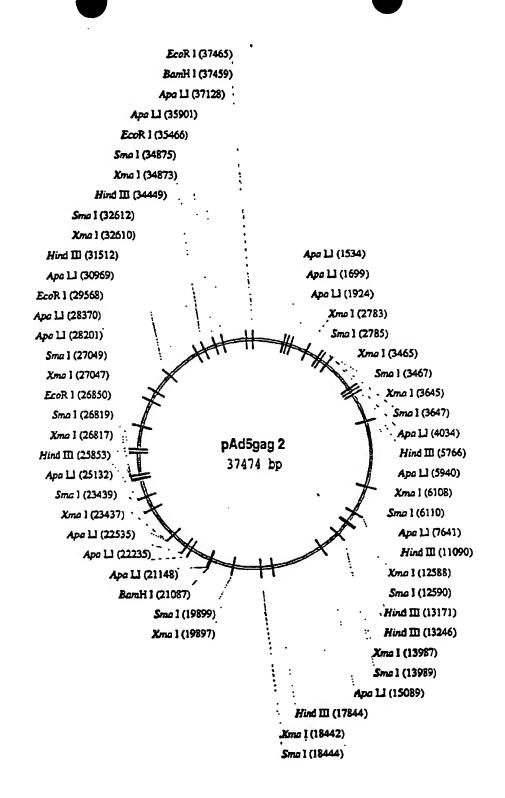


Figure 14

מכאמארטנות דידיפיינאכיודי מסכמנוסממכי

THANKINGAAT ATGATAATGA

TATTINGAL

TAATATACCT

PTCTTAATTA ACATCATCAA

ACCAGANI 'AA

ATTOROGOGO TAACTCCTCC ACCTCCAGO TOGAGGICCC

COCACOTITO

GAGCTCGGGA

CATACCCCACTC CITTIANGGALA CACTICCTOT ANATHOTICCCA

CCTCCCTCTA

CCAGATOM

ATTOTOTOCAGA

GANCTACCCC

CAGETICITY TICCAROTCO ICCARCARO ACCONTINCOS ITYMASITYAS ICCARARAT CITUATURAS

TOTAL MORE

TAACACGTCT

CCACCTCTTC

CONTRACTOR

CTTATANCTA AACTICACK

CACACCTONT

TOGGACATGA ARRETRICTAR

CHCTYSCACCA

ACCEPTACE

AGACTCCCCA

CGACCTCTOO

TOCCACCOGA CACAGTOGCT GTGTCACCGA ANGOCCCAGC

NANCGACACT

CCCTOTACAA

1501

COCACATGTT

OTCCARRANG

1601

CANCATTICAT

CLIANATIAGGC

CCTOGAGAAG

GOTCTACCAC

GNCACTORY C CCTATAGEAT GTANTCAAGT AATCACCTAT TTACTOCATA TCATGTAGAT CCATRICTICAC CTCTGGGCCU" מכונטעטעטעטט ריאאאטפאייו CHCHONOCH CATTAGETICA ATAGTATACT AGTACATOTA ACIDICACIONAL PARTICIPATOR PART ACCACACACACC CCACCACATO CACCTGAGGT **FATCATATY** CCATATCAT TCTCCACCC **AGAGGTTAGG GETACKEGT** ACTOCANANA **OCCATITIOS** COTTTACGTO CCAANTCCAC TACGETGEAT ATCCAACATA ATTACGGGGT TAATGCCCCA TOACGTCAAT ACTOCAGITA ACATCAAGTG TOTACTTCAC CCTACTTOGC GGATGAACCG DATTTCCAAD CTAMAGGTTC COTTTACCCO CTCCATAGAA CACADACTO CAGGTATCTT AGGGCTTCTG TCCCGAAGAC CCTCCAGGGA COACCTCCCT AGGCTCTGAG TCCGAGACTC وحورسودروه TCACOFFITT COGTINAAGC GCAMTGGGC CATTICTARAC GGACTITICAC CCTCAMCTO ATAGTAATCA TACCCTIGANA CCCCATTGAC CTGTTTTGAC TRANSATICTAC CATGOGIGCT GTACCCACGA ATTICITITICS TANCACACCC CCCTGCAAAC CTCCCNNNG CACCOTTTTC GTAAGATTTG ATCCATTIGCA TAGGTAACGT CCCCGCCCAT ACTIVOCAGE TGAACCGTCA GACTCACOO CTCAGTGCCC COCCTANCTO TATCATTAGE GROCCOCTA ANACACTRICA ATTOOGNCTTT ACKICCICIOG 2020022022 CTACTTATTA GATCAATAAT GCCCAACGAC TANACTOCCC ATTITISACGO CGGTARGTGC ACTCTAGATO CCTAAAGCAC TCACACCGCC TIGIGIACAT TCACTCCCTA CCCATTCACCT **TCCCCGCCCC** CUCCCCCCCC ATACCCOUNT ANCANCTECE TTGTTGAGGC GCCATCCACG CGATTTCGTG CTCCAGCCCT CATCATANCEGA COGGTTGCTG ACATGACCTT TGTACTGGAA TATCCCCANA מרככאמכיוכ ACKLEACCICAT COCAACACTA CATTOGNACTA COGNITICCCC GTACTAACAG ATATTTGTCT ATANTANTA TCATTATTCA CACCCIACTED CINTITACIA CATAMITEC ATTGGTTTTGG TITITACAGCA GCCTTGAAGAC COGNICITY CACCATTACTIC ACCARCTACAA TYTHYARTE CCTCOCCCAG CITAMITTICG THTANACAGA TATTATTA ACTAATAACT TATGCCCAGT ATACCCCTICA TACCCACACC AAAATISTOGIT ACTINISHIN TWITATTACT CATTFFAAAACC CHARCETTRACE AACACATGTA CATTGACGIC AATGGGTGAA GTTTAGTGAA CCGTCAGATC CCAGATATAT TCGTCTCGAG CAANTCACTT GGCAGTCTAG CHARCETTOC GCCTANGGG CAMITICAAA TACAACTGTA TTACCGGGG CAGTACATCA CCCTCAAACG いっしょうしょうしょう CCACCGTTCT CCAGCCAGAT GGATGTTAGTA ATMOTOCOTA COTICATOR ANTRACCICAC TTACCCACCT CCCCTACCCAT CCCGACCGTA CTCATKITAGE COGACTITICC ATCTTCACAT ACTESTICATION CCTACAACAT TATICAL STATES GTTTTGGCAC CAMATCAAC CGACTCCGGA TETCAGGGGT ACACAATGAG CCCCCCCTCAAA **GTANTGGCGG** ACTITACOGITA TCAATGCCAT OFMCTGCAG CTAMATACCC CATTTACCOS **CCCCTTTTCC** CCCCANANCC GTTTTAGTTG **GCTRIABGRICT** ATANANCCTA TriATriTTYICA CCANANTCCG GOCCCAGTIT ACTACAACGT **GOTTITARGG** TUTCHTACTO CATTACCGCC AGGGACTITIC CCCTTGCCAC AGAAGATCAG TCTTCTAGTC OCTACCACTA CAMACCCITO GCTOGAGACC CCATCGTGAT THICKNOTT AGTACAGGTT **GCGFTACATA** COCMATGTAT TECCTOMAG GTCAATGACG ACCAGAGETE ATTATATATA האכסהאתידה CCCCCTTCAC ATTITION TAMAGERIC TCANTANTIT ACTIVITION MAGRICICA TCATCTCCAA CAGTTACTOC ATTOCOGITA GACAAGTGGG ACCCTODOCT CCTTCACTGT AGAGTCCACA ATCCACTTCC TAACGCCAAT CCCTATTCAC GOCATAACTO ATCCCTATTA ATCCCACTIT TACCCTCAAA GENCTATATA CHECOGGGGC GAGGCGCCCG CTGTTCACCC TRETACTACTE GTAGTAGNET CATCATCACA CORACTICACA TICACTITAG TCTCAGGIGT TTATATTGGC AATATAACCG TACCTCAAGG TACCGATAAT NACTONNATC CCGATCCAGC TTTOCTOTON ACCACTCGAC CACCOCCTOAC CCACATGTOF CANTARGAGG ATATGTACAT FACCCATAT ATCCCCCTATA GITCCCATAG CARGOSTATC GTTCATGCGG COTATTACTC ATTOACCICA **PACTGCAGT** TACCOTCOCA ATGCCACCCT DOCTROCTEG RECITEDADCTG CCCCCACTG COTOTACACA GTCCACAAAA TATACATOTA CAAGTACGCC CCATAATCAG ANCHITANT CTTAITCTCC CAGGIGITIT 1001 1101 1201 1401 901 1301 501 601 701 HOI 101 301 401 201

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1701	CACCAGGCCA	TCTCCCCCC	CTCCCTCAAT	CCCTVAXCTCA	Acceptations, Accept	CHARTICGES MANAGASAC	THETECTE	ARCITE AT CCC	CATOTICICI	GCCCTGTCTG
1801	ACCACGGTG				ACTIVE STREETS	CATACTOCGAC	CCATCCACAT	GCTGAAGGAG	ACCATCAATG	ARRANGOCITY TECTYTERAL:
1901	TCACTCCCAC				מטיינוניוניני	ACATY:ACCION	CKCCAGGGAC	TCTGACATTG	CTORCACCAC	CHCCMCCT-
2001	CAGGAGCAGA				CACACCCCT		ACCACCTAGE TCCACCTAGE	TCCTOSCCT AGGACCCGGA	GACCAGGATT	GREACTART
2101	ACTCCCCCAC				CCTCCCCTTC	AGGCACTATG TCCCTGATAC	TGGACAGGTT	CTACAAGACC	CTGAGGGCTG	AGCANGCCT"
2201	CCAGGAGGTG	ANGA			CACAATGCCA		CANGACCATE	CTGAAGGCCC	TOGRECE TOC ACCEGGONED	FOCCACCETO ACGGTGGGAV
2301	GAGGAGATGA				CASTUTUCOS	CAGGGTTGCTG	GCTGAGTCCA	TOTCCCAGGT	GACCAACTCC	GCCACCATC, CGGTGGTAGT
2401	TCATCCAGAG		ACCINCACA	GGAAGACAGE	CATCACGAAG	AACTISTICCA	AGOTGGGCCA	CATTGCCANG	AACTGTAGGG TTGACATCCC	CCCCTANGAN.
2501	GAAGGGCTGC	TOGAROTOTO	GCANOGARRO	CCACCAGATO	MOUNTACA	ATGAGAGGCA TACTCTCCGT	GCCCACTIC CCGGTTGAAG	CTGGGGGAAA	TCTGGCCCTC AGACCGGGAG	CCNCNAGOCA: GOTOTTCCUT
2601	AGGCCTGGCA				CCCCTCCCGA	CCTCAGGAAG	AGGTTTCORD TCCAAACCCC	ACCACTOTICES	CACCCCCAGC	CALUANGCAR
,										Marine American American
2701	ACCCATTGA	CANDGAGCTO	TACCCCCTRIG ATTCCGGGGACC	CCTCCCTOAG	CAGGGACAAA	CCCSITTCXCTTSG	CCTCCTCCCA	CATTITATIT	COCCCCTC	TAGACGACA
2801	CCHICTAGE	OCCAOCCATC COGPCOGTAG	TOPPOTITION ACANCAVANO	CCCTCCCCC	TCCC TTCC TT	GACCCTGGAA	GENCEACTE CCACGGTGAG	CCACTGTCCT	TTCCTAATAA AAGGATTATT	ANTCACCANA TTACTCCTTT
2901	TTOCATCOCA	TTGTCTGAGT	AGGTGTCATT TCCACAGTAA	CTATTCTCACG GATAAGACCC	CCCACCCCAC	CCCGTCCTGT	CCAACCTOTA	GCATTGGGAA CCTAACCCIT	GACAATAGCA	GENTACTED
				Asci		Value	AGCCCCCAA	BCABTATATA	Acendocacer	CHTATGTAGT
3001	CCTACGCCAC	CCGRGATACC		CCCATCACCAC CCCCCATCAC	TTTACACACC	CCCACCOAAT		TCTTATATAT	3	GANTACATCA
3101	THETATETE	TTTTGCAGCA AAAACGTCGT	COCCOCCACC	CCATYANGCAC GGTACTCCTG	CAACTECTITE	CTACCITYCT	TOTAL SACACTECAS	ATATTTCACA TATAAACTOT		CCCCATAGAC GGCGTACCC11
3201	CCCCACCCA	CACANATGIGA	ACCCTAGGTC	CATHGARGGE	GCGGGGGGACAGG	TOTICOTOTIANA	CTCTACTACC	TRACCTACG	ACACCCTCTC TCTCCCACAG	TCGNACGCCG ACCTTGCGGC

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3301	TOGAGACTO	CARCCTCCGC	CACCICITICA		בראניניקינה	CATCH MACAC	ACTIVACT TTO	GNACACTC	CCCCCTTGCA	AACAK:TGCAG TTCTTCACGTC	
3401	CHICCCOFFIC			TCACCCCCATCT ACTRICCCCACA		THE ANTICITY AND AND AND AND AND AND AND AND AND AND	TGACCCCCARGA ACTOGRACCCT	ACTTANTOTC TGANTTACAG	CAANGAGTCG	ACK:TK:TTK##A TCGACAACH "F	
3501	TCTCCCCCAG AGACGCGCTC	GECCAMBAC	CCCTYTAAGGC	PRUMECOUP	CCCAATTACKS GRATTACKCC	TITIMMENT	ANNTANAMA	CCAGACTCTG OCTCTGAGAC	TTRESTITE NANCETANNE	GATCAAGCAA CTAGTTCGI I	
3601	GIGHCTFOCT		ACCCAAAAC	C G C G C G C C C C C C C C C C C C C C	ARGCCCTARA TCCGGGCCCT	CCAGCIATICT	CONTCOTTGA	CCCAGGACAC	TATTTTTTCC	ACCTGCACCA	
3701	AAAGCTGACT	CTOGATOTIC	AGATACATGG	CCATARCEC	CACACACACC	TYSAGGTAGG ACCTICATCG	NCCACTFICAG TCCTCACGTC	ACCTICATGC TCGAAGTACG	TOCOGOOTOO ACOCCCACC	rcticta gat Acaacateta	
3801	GATCCAGTCG	TAGCAGGAGC ATCGTCCTCG	CCACCITICAC	CACGGATTTT	ATCHETTICA TACAGAMAGT	GATCGTTCGA	GATTRICCAGG	GCCAGGCCCT CCGTCCGGGA	TCOTOTANOT	GITTACAAN: Caaaighthe	
1901	CCCATTCCA		CATACGTORIC	GATATRAGAT CTATACTRTA	GCATCTTAGA	CHSTATTITT	ACCTACCGAT	TGTTCCCAGC ACAAGGGTCG	CATATCCCTC	CORRESTIVIA OCCULTAMENT	
4001	TOTTOTOCAG ACACACOTC	** AACCACCAGE	ACAGTGTATC TGTCACATAG	CCCACCTICAA	GGGANATTIG	TCATGTAGCT	TAGANGGANA	TCCGTCGAAG ACGCACCTTC	NACTTGGAGA TTGAACCTCT	CCCCTTGTVI	
4101	ACCTCCAAGA		ATTCCTCCAT	AATGATGGGA TTACTACCGT	ATTAPACTORY TATCCOGGIG	CCCACCACGGC	CTTOCCGCAAG	ATATHTCTGG TATAAAGACC	CHACTCATTO	CAGENTEANC CAGENTE	
4201	TOTTCCAGGA	TOAGATEGIC ACTETAGEAG	ATAXXCATT TATCCXSTAA	TITACAAAGC	GCCCCCCCCTC	CCACCGTCTG	TOCCOTATAA	TOGITICCATC	COCCCCAGG	GCGTAGTTA CGCATCAA'N:	
4301	CCTCACACAT		CACCCTTIGA	CTTCAGATOG CAAGTCTACC	OCCUPATION CONTRACTOR COCCUPATION COCCUPAT	TCTACCTGCG AGATGGACCC	GGGCGATESAA CCCCCCTACTT	GAAAACOOTT	TCCOGOGTAG AGGCCCCATC	OCCIOCIMENTO CCCICTAGING PSI	
4401	CTCGGAAGAA GACCCTTCTT Pst	AGCAGGITCC TCGTCCAAGG	TOACCACCTO ACTCGTCGAC		CCACTFACCS CACCCATAS GCCCSTNANT OCTSIANTECC CACCCATCTA		CACACCTATT	ACCOCCICCA	ACTOOTAGIT TGACCATCAA	AAGAGACTU: FTCTCTCGAC	
4501	CAGCTGCCCT	CATCCCTGAG	CAGGGGGGCC	ACTTCGTTAA TGAAGCAATT	GCATGTCCCT CGTACAGAGA	GACTCATATG	THTTCCCTGA ANANGGACT SniM	CCAAATCCGC	CAGAAGGCGC GTCTTCCGCG	TCCCCCCCCA ACCCCCCCCC	
4601	OCCUPANCAS CRCTATCOTC	**************************************	CHICCHTICA	TTTTCANCYO AAAAGTTCEC	TTTGAGAGGG	Treathrand Available Available			CCANGCAGTT	CCAGGCGGTC	
4701	CCACAGCTCG	GICACCTOCT	CTACGGCATC	TCGATCCAGC ACCTAGCTCC	ATATCTCCTC TATAGACTAG	CMANACACA				CCCACGAGGA	
4801	CCAGACGOGC	CACCCACTATO GICCCACTAC	TCT-TTCCACG AGANAGGTGC	המאשראים הכמכהבכבים	CCTCTCTTCAGG	CATCAGACCC	TCACCACTT	CCCACCACA	CCCCGACGC	CCACCCCT"	

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4901	CCACCCCANC	AGACTIONTEC TCCGACCAGG	TCCTCOTCCT ACTACCACGA	GAAGACA-TIVIC CTTRCGCIACIS	CCCACACAC	CITTORITACITO	CCCCACTEND	CATTICACCA	TCCACAGTAT	GICCAGACAC	
2001	TCCGCGCGT	GGCCCTFGGC			Antichi CrecA	CTIMITABLING	TOCACACTT	TOAGGGGGTA	GAGCTHZZZ	GCTAFAAATA	
5101	ACCURACION A	CCGCCCCCC			ורבוש ואירנים	CATTER COL	M.V.I.C.IVIVA	שר וככנסנשו	CICCAMICE.	ANACTACHT	
	GCTAAGGC	CCICATCCGT		TCCGGGGCGT	CTYCCAGAGC	GTAAGGTGCT	COCICCACTC	GAGACCGGCA	AGCCCCAGTT TT	TITGGTCCA!	
5201	TCCCCATGC	THITTGATGE	GHINCTIFACC	TCTGATTICC	ATYANGCOGT	GICCACGCIC	GCTCACCGAAA	AGGCTGTCCG	TOTOCCCGTA	TOTOCCCGIA TACAGACTIVE	
	AGGGGGTAEG	AAAAACTACO	CAAAGAATTOG	AGACCAAAGG	TACTURECA	CARGICCGAG	CCACTICCTIT	TCCGACAGGC	ACAGGGGCAT	ATGICTGAN:	
5301	AGAGGCCTOT	CCTCCAGCO	TGTTCCTCCGG	TCCTCCTCGT	ATAGMACTC	COACCACTOT	CACACAAAGG	CTCGCUTCCA	GOCCAGCACG	AAGGAGGCTA	
5401	AGTOGGAGGG	OTACCOCICO	TTOTCCACTA		TOTACHOCAGG	_	ACATGTCGCC	CICITOGGCA	TCANGGAAGO	TOATTOSTE	
	TCACCCTCCC	CATCGCCAGC	AACAGGTGAT	CCCCCAGGTG	Nacrangetec	CACACTTCTG	TOTACAGOOG		Agriccince	ACTANCCAM	
5501	GTAGGTGTAG CATCCACATC	OCCACOTOAC COGTOCACTO	CCCCACAAGG	TGANGROPHS ACTTCCCCCC	CTATAAAACG	GACTORDECTC	CCCAACCAGG	TCACTCTCTT AGTGAGAGAA	CCCCATCCCT	GTCTCCOAGO	
5601	OCCAGCIGIT	COCCICACTA	CTCCCTCTGA	AAAGCGGGCA	TCACTTCTGC	GCTAAGATTG	TCAGTTTCCA	AAAACGAGGA	COATTICATA	PPCACCAGO"	
		, cecement		101120111	Sanous and		Hingel				
5701	CCCCCCACTA	OCCTITIONOO COGNAACTCC	Gracecear CACCGGGGTA	CCARCTURATIC	AGAAAAGACA	ATCTTTTEST		CCACCOTTES C	GACCEGTAGA	CCCCCARCCT	
1002	· Page age age	- EMERCANA CARD	·		Pwd		CECCAPERTY	Actorities	ATTICIONALISE	AACTOCACTOR :	
1000	OTCOTTOAAC		COTCCCAAAC	CANAAACAGC	CCTACCCCC		GCCCTACAAA		TAAGCCCCC	TIGGGTGGCJ	
5901	CATTCOOGNA	AGACOOTGOT TETGCCACCA	GCGCTCGTCG CGCGAGCAGC	GOCACCAGGT	GCACFICTICCA CGTOTGCAST	ACCICCOCITIO TEXECOCEAAC	TOCAGGGTGA	CAAGGTCAAC	OCTOGREGET COACCACCOA	ACCTCTCCOT TOCAGAGGC	
6001	GTAGOCOCTC	GATEGOTECAG	CAGAGGCGGC	CUCCCTRACO	CCACCAGAAT	GACCATCCC	GGTCTAGCTG	CONCINCATOR	GOCCCCAGAC	CCTCCACCACT	
6101	AAAGACCCCG	CCCTCCTCC	OCCOUNT COA	GTASTCTATC	THECATECTE	GCAACTCTAG COTTCAGATC	CCCCTCCTCC	CATCCCCGG	COCCANGCEC	GCCCTCGTAT	
6201	CCCAACTCAC	CCCCTGGGGT	TOCCATOCOC ACCGTACCCC	TRECTION ACCENCING	CGRACTA	CATCCCCAA	ATCECTTANA TACAGCATTT	CCTAGAGGGG GCATCTCCCC	CTCTCTCAGT	attecaagat Taaggtteta	
6301	ATOTAGOOTA	OCATCTTCCA COTAGNAGOT	CCCCCTATA	TRACIACIAC AC ACCACACAC	GTANTCHTAT CATTACE:ATA	ACTION CACC	MAYCHOCHOCHO TECETEGETE		CCGAGGITGC	TACGGGGGGT: - ATTACCCGCC	
6401	CTOCTCTOCT	COGAAGACTA	TCTCCCTCAA AGACCCAACTT	CTACCCTACA	GALTTRECATE	ATATACTAGE TATACCAACC	ACCCACCTTC 1	ACCITICAACC 1	NCCCCAGACA (GAGACTTACI: CTCTCCATATA:	

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TCCTTGATGA AGGAACTACT COTCGACCT*	OCALCOCKON:	GAAGGCCTV"	AGCAAAAARTT TCGTT!!TTCA	TGCGGAAGGT. ACGCCTTCCT	CANGAAGCG OTTCTTV:ACT	COTTCTACTIC	AAAAAAGACC	ATCTCCGCCG TAGAGGCGGC	PCGACGCATA	CCGAACACTC	OAAGCAGAGT CTTCGTCTCA	ACCOPTICATY: TRECACCENS	TGGTCTGGAG ACCAGACCTC	ттеслявайс лаватесеся	TCCTTGGATY; AGGNAGCTAY
ACCA COTO			-		-									TTCC NAGO	AGGN
GTCCAGAGTT CAGGTCCCAA ATCGGAAACC	TAGCCTTTGG	CONCOCCCC	CHCCHCCCAG	MCGCACACT	ACATTICANG	CCCOOTCAGA	CCTATOCCA	CTAGAGGCTC	AAAGAGACGC TTTCTCTGCG		ACCTGACGAC COCOCACANO TOCACTGCTG GCGCGTGTTC XH01	CTRACTOCTC GARGODAGTT GACCGACGAG CTCCCCTCAA	CAGCTGTCCA	GATACCTAAT TYCCANGGRC CTATGGATTA AAGGTCCCCG	COCOOCITATIO
CCCCCCTCATA CCCCCCCTCAT		-						OCCIO COCCIO POR COCCIO COCCIO POR COCIO POR COCCIO OR COCCIO OR COCCIO POR C	CGTARGTGAC				GCGCAGATGG CGCGTCTACC	AGATECAGGE TETAGGIECA	GOCAGTOGOC
Treachereta Acotorachant Goterforea			TCAGTGTCGT AGTCACAGCA					TAGGACTOGG	GACACATCACAT	TCTCCTGAAA	CACGAGGTTG GTGCTCCAAC	CCTTGACCGT	TENCANCATO ACTOTTOTAG	GACCAGTCAG GOCOCCAGCT CTGCCCAGTC CCGCGCCGA Kod	GALTAINSTIN CENTROCATICO GOCAGIOGOS COCOGOGISTIO ECETITICANES ETIGATACENE CAGOCOCOCAGE AGGINECTANES ETIGATACENES CAGOCOCOCAGE AGGINECTANES ETIGATACENES CAGOCOCOCAGE AGGINECTANES ETIGATACENES CAGOCOCOCAGE AGGINECTANES ETIGATACENES CAGOCOCOCAGE AGGINECTANES CAGOCOCAGE AGGINECTANES CAGOCOCACAGE AGGINECTANES CAGOCOCAGE OCAGOCOCAGO AGGINECTANES CAGOCOCAGOCOCAGO AGGINECTANES CAGOCOCAGOCOCAGO AGGINECTANES CAGOCOCAGOCOCAGO AGGINECTANES CAGOCOCAGOCOCAGOCOCAGO AGGINECTANES CAGOCOCA
ביניפיריאלוגל ביניפיריאקיוגל אארייצידיאריאל		ALK: ATCCCTT TCCTTAGGGAA	CATANACTIC	AAGAGTATUT TTUTCATAGA		-	ARATCATCHE TECANCOG	CONVINCE	CUANGTATAG	TOCCTATTGA ACCGATAACT	CATGTAGGAC	CCCACCAACA		GACCAGTCAG CTCCCCAGTC Kprd	
TCACCAGCTC ACTOSTICIAG		#CCTAGGGGG	TYGACKTETAL TYG	CTGTAGCAAC		AGGTGAGCTC TCCACTCGAG	TAGCATTAGE ATTCGTANGG	TCCCATCCAA	AGGCCCCCAT	ATTOCARTAGE TANCETECTE	ACCINCTANCE	CTTCTACTTC		· ACCTCOCATA	ANGICIANTE CECTICIANOS TECÇGEGING GORDICARO
		GTTGACGCCCC CAACTGCCCGG	ACCATGACTT TOTACTGAA	CCCCTTCCA	CHCCCCCCCC	AGTICCTCGT TCAAGGAGGA	CACTROCCOSTA	Trecendend	TOCTTOCCAA	CCCGCCACCA		CCCACCACCA		CTCCACCITT GACGTCCAAA	
_		TGTAGAACTG ACATCTTGAC	GCTGTCCCTG CCACAGGGAC	GCTAAACCGT	TOTTAATTAC ACAATTAATG	CANTITITIA	CTCCACAGGT	CGCCCAGAAC		AACTGGATCT	CACOCOTCAT	TCCCCACTT		GEOOGAGETE CGCCCTEGAG	GOCTTOCANG
CGAAGGAGGC	ATCCTGTCCC TAGGACAGGG	GAGGCTAGGA	TCACCCCAAA ACTCGCGTTT	TTTOCAACGC AAACCTTOCO	TCGGAACGGT AGCCTTGCCA	TCATCOAAGO ACTACCTTCC	CHCCAATGAG	TACANGGTAA		CATCCCACC GATCCCGAAG		מכככבובפכב		GPCAGGTCAG	TOSTICISTOS COSCOTCGAT GOCTTGCANO ACCAACCACC GCCCCAGCTA CCGAACGTTC
GCOTCACGCA	TOTCATACTT	CCAACOCTAA	GAGCHOTOGO	CCGTGCGCTT	TCCCGGCACC AGGGCCGTGG	CCCTACGGGA	CCACCITICS	CONCATOCAG	AACTICATOA	CATGCCAGCC	GTOCTOSCTT CACGACCGAA	GOCAATTTOA	CCTOCTOCTO	CPCCCGCGGC	TOCTTOGTOG
6501	6601	6701	6801	6901	7001	7101	7201	7301	7401	7501	7601	1101	7801	1901	8001

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8101	NTOCATCTAA		-				CGCGAGAGG			GCGCGCGCAX	
	TACGTAGATT	TTCCCCACTO	COCCUTATIVE	ののはいいというの	AND THE PARTY OF THE				いっという	רוברנהיישררייי	
8201	ASSAGETAGE		_	עכניטאעניניניא	r:Mtxtxp:rg	הדומאוכזיינ		accretacer.	CAACACCACC	ממכנוטייוניא	
	TCCTCGACCA	COVCOCCCCC	ATCCAACGAC	COCTACA:CCT	מישעיייניים	CAACTAGACG	N TINGACCG	CGGNGNCGCA	cricionoc	CCGCCCACT	
8301	GCTTOANCCT	GAAAGAGAGT	TECHCACANT	CANTITURGE	CHECHICACG	فالروبال لأعار	CICANANTICTIC	CTYSCACGTCT	CCTGAGTTGT	CTTCMTAGG:	
	CGAACTTOGA	CTITICACTICA	AGCTGTCTTA	GTTAMGUCA	CAG'AAC'IGC	כניניניניניערכני	CCTITTAGAG	GACOTOCAGA	GCACTCAACA	GMCTATCC":	
				Ilga .	4119						
8401	GATCTCOOCC	ATGAACTOCT	CCATCTCTTC	CTCCTCCAGA	CHEXTHEREAGA TETECHOGITIC	ניטיניווישכוני	CACOGINGES	GCGAGGTCGT	TOGRANTOCO	CCCCATGA! "	
	CTAGAGCCGG	TACTTGACGA	OCTACACAAG	GARTACCTUT	AGAMAGGGAG	GCCCAGCCAG	CINCCACCCC	COCTCCAGCA	ACCITITACGE	CCCITIACTU:	
8501	TOCCHOAGO	COTTORGECC	Tecencome	CAGACGCAGC	TETTAGACCIAC	מנכננכונונו	GCATCGCCAG	COCGCATGAC	CACCTGCGCG	ACATTICACT. "	
	ACCENTINCE	GCAACTCCOG	AGGGAGCAAG	GTCTGCGCCCT	ACATRCTRGGTG	CHASCIASIANCE	CCTAGCCCCC	GCGCGTACTG	gradacacac	TCT/MCTCR.	
8601	CCACGTOCCG	GOCGAAGACG	OCCIAOTITC	GCAGGCGCTG	ANAGAGGTAG	TINGACOCONOS	naccoanting	TTCTGCCACG	AACIBAGTACA	TANCCCARC	
	CONCACOCC	CCGCTTCTGC	CGCATCAAAG	COTCCGCGAC	TITCICCATC	AACTCCCACC	ACCOCCACAC	AAGACOGTOC	TTCTTCATGT	ATTGGGTC(X)	•
		ŭ	EcoRiv								
8701	recentering		GATTEOTTGA TATECECCAA	OCCUTCANOG	CCCTCCATOR	הכדרהדאמא	GTCCACGGCG	ANCITICADADA	ACTORGAGIT	GOCCCCCAC	
	ACCOPTICCAC	CTRACEMENT	ATAGGGGGTT	CCGCAGTTCC	OCGAGGTACC	GCAGCATCTT	CAGGTGCCGC	TYCANCTITY	TOACCCTCAA	כפכפניפפבוני	
8801	ACCOPTANCT	CENTECTICEAG	AAGACGGATG	ADCITICATION	CAGTGTCCCG	CACCTORNIC	TCANAGGCTA	CAGGGGGCCTC	TICHCFICT	TCAATCTCE:	
	TOCCAATTOA			TCGAGCCGCT	GTCACAGCGC	GTCGAGCGCG	ACTITICCGAT	GTCCCCCGGAG	AAGAAGAAGA	ACTTAGAGGA	
									Sall	3	
8901	CTTCCATAG	GOCCTCCCCT	refrencer	CTOCCOCCG	TEXTAGENCERS	GCGACACGC	GGCGACGACG	OCOCACCOGO		CANAGEGETE	
	GAAGGTATTC	CCOCA	AGAAGAAGAA	GACCOCCOCC	AncenceTeers	CCCTGTGCCG	CONTRICTION	COCGROGECC	TOCCOCCAGOT	GPTRACAGAG	
9001	GATCATCTCC	CCCCCCCCAC	GCCCATIRET	CHECKINIACE	Granana	TUTCGCGGG	GCACAGTTOS		CCOTCATGTC	CCCASTANCE	
	CTAOTAGAGO	OCCCCCCTC	CCCCCTACCA	GAGCCACTOC	CGCGCCCATCA	AGACACACCC	CCCCTCAACC	דוכדוסכסמכס	OCCUPTACAD	OCCCAATACK:	
9101	GTTGGCGGGG	COCTOCCATO	COCCAGGGAT	ACCGCCCTAA	CGANTICATET	CAACAATTAT	Transmit		GACCIACCTO	ACCCAMPERT	
	CANCEGEEEC	CCCACCGTAC	GCCGTCCCTA	TOCCCCCCATT	GCTACGTAGA	GFTGFTMCA	ACACATCCAT	GAGGCGGCGG	CICCCTOCAC	TCGCTCAGG	
			X								
9201	CATCUACCOO	CATCGACCOG ATCGGAAAC	CTCTCGARAM	ACKICCITCTAA	CCAGTCACAG					occences:	
	GTAGCTOGCC	TAGCCTTTTG	GAGAGCTCTT	TCCCCAGATT	GGTCAGTGTC	AGCGTTCCAT	þ	GCACCGCCCG	CCCICCCCC	CCOCCAGCCC	
							Sati				
9301	orrorreto	OCCIONACTOR	TOCTGATGAT	GTANTTAMG	TAGRECIATION	TYAGACTGCG		AGAAGCACCA	marcer 1000	TCCCACCTCC	
				CATTANTITIC	ATCCTACAGA	ACTICTOCOGO	CTACCAGCTO	Terrester	ACAGGNACCC	ACCCCCCACACC	
9401	TOAATGCCCA	OOCOOTCOOC	CATTACCCCAG	General	GACATCHACH	CACATHCTTAG	TAGTAGETT	GCATGAGCCT	TICTACCOCC	ACTICETICE	
	ACTTACCCG		GTACCOCCTC	CGAAGCAAAA	CTCTARCCCOC	CHECAGAMAC	ATCATCACAA	COTACHEGGA	AAGATGGCCO	TGANGNAGA:	
9501	Creerveer	TIGHTCTIOCA	TCTCTTGCAT	CTATCACTOC	DESCRIPTION	CACTTIVAZIC	GTAGGERGE		CCCATGCOTO	TGACCCCGA.	
	CACCAACCAC		AGAGAACGTA	GATAXXGACO	כנגאניאכניטכ	CTCAAACTTO	נאונכאונפנ	CCCACACACTCA	COCTACOCAC	ACTROOPER	
1096	CCCCTCATC	COCTCAACCA	GRACTAGGTC	CATC: ACAACG	CCCTTCCCTA	ATAROSCITO	CHICACCTOC	-	ACTORANGE	APCCATCHECT.	
	COOCCACTAG		CCCCATCCAG	CCCCTGTTGC	OCCARCCRAT	TATACCAGAC	CACCTCCALC	CACTCCCATC	TCACCTTCAG	Thagthchay	

ACCCACATOR:				CCCACCACCT:		CGTAATTCA(CCCCANG		CCCCAACTIX		COGOCTICCTC	ACTICOGCTO COCOLTIGGE Handly	AMOUTITIT COMMITTEE	
CTCCTAGAGC									-					
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TTAACGGTCT AATTGCCAGA	ACCUSTRIC GETATGGGGGGGTG TXXTICATIN CCATAGGGGTG	CCCCTACTAT ACCCATCTAC	TGCTCCATTAG ACGMGGTACC							AGGAGGGCGA	-	THICTICICIC		GANCATTCG GCGTTGCTAA GCGGACGATT
NACGGACTAG TTGGTC	ACCIVATINCT C TXTTICATIN (CCCCTACTAT	CCCCTTTTTC	CTTCCTKAGT	CCCCCTTGTCG			AGCCCCTTTT TCGCGGAAAA		CHICACTTON	TCCCTCCTTA ACCCCCCT	Trigensagens Agensetense		ATT NEIKERER TAGACACCT ATTCACCATAT TAACTCCCTA
AGTHERICAT AACHAAGAACTAG TCAACCCXITA TTGCCTGGTC		GOCCECTAGA AGGITGIANT	TOTTGCGCAC		CGATTACCAC	TTTTGTCAC AAAACCGGTG	AGTICECCEGE TEAGCGCCCT	AACAGGGAGG TTGTCCCTGC	CATOCAGOGO GTACCTCCCG	CCCCCACTAC	GAGGCGTACG CTCCGCATGC	ATCCCCTCAA TACCCCCTCACTT		GRACTIONTIC CCTGACTM:0 ACANTSARTIC TGTTRICTCCO
GREATHCACK	CCTTACTICATT GCATCACACA Refit	CCCCTCTAGA	CCCTTCCAGA	AGAGCCHGTA	GHGATCCATG	GCGCTAGCTT	CCAAGGGTTG GGTTCCCAAC	TTCCTCCGGA	CACCCCCAGA	SCACAGGGGGC CCAGGGGGGGGGGGGGGGGGGGGGGGGG	TOATACGCGT ACTATGCGCA	GAGCTOCAAC CTCCAACACCA		GGTGGCTATA CCACCCATAT CACAGEATATA GTGTCGTCCC
	GAGTCAAATA	GCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	OTCHCGGAAGG CAGCGCCTGC	OTOCANAGO CACOTITICO	OCCUPACING COCCUPACIONS	CCCCCCACCA	CCCANTRARA	CCCTTCCAAA	AGAGCAAGAG PCTCGFTCFC	GMCCCCCCC	AGCTGAAAGCG TCGACTTCGC	CCCACCCCC	CACGTGGCRG GTGCACCGCC	CGCCGCTCCT GCCCGCTCCT TATAGTGCAG ATATCACGTC
DCTATOCCC COTATTGATG CCATACCCCG CCACAACTAC Whole	GTAAGCCC' CATTCGGG	ARRESTORCES TECEACEGGE	CCCCCCCCTTF CCCCCCCTTF Xbs1	88	CCCGTATCCG	Trccagede AAGGTCCGCG	TOTAGCCGGA ACATCGCCCT	TOCANGACCC	AGCAGCGGCA TCGTCGCCGT	TOGTOATTAC			cococococa ococococat	ACCCTHOTOG TCCGAACACC ACCTGTTCCT TCGACAAGGA
ACAAACOST OCTATOCOCC CONSTIGATO TOTTTCGCCA CCATACOCOG OCACAACTAC NAME OF THE OCACAACTAC	TCACACCCCA	CCCCGTCGCA		AATCOTTOAC TTAGCAACTG	GOOTTCGAGC CCCAAGCTCG	THOOCTHCC AMCCGAAGG	COACCOACC	CICCCCOTCA	CCCCCTCCTC	COCCACCACA	TEAGCGGCAC ACTCGCCGTG	ATGCGGGATC TACGCCCTAG	GCTAATCAGG	CCACGTGCGT GOTGCACGCA CTCATGGCGC GAGTACCGCG
9701	9801	9901	10001	10101	10201	10301	10401	10501	10601	10701	10801	10901	11001	11101

11301

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1301	TCCATTTCAT		AGAGTATAG	אימידיאיראהה ואימידיאידידה						Traccion	
	AGCTANACTA		TITICIAGGAC GICINCOTATO	ACCIACITATION	CLUTHITANC	עייהיארייניאני	אידונינאתמים	CCCCTACTTO		MATCHASACTY	
1071	CABCITITATA		CANCELARGA TATALLATAL	CCTTACGIT	CHUNKINGA	ACCIDENTAL O	ניאבניניעניטבעי	TTCTACATGC	CCATCCCCCT	CAAGGGTGCT .	
105	GFTCAAATG		ATATECTATE	COCAATOCAA			CTACKTECKE	AAGATGTACG	CCTACCCCCA	CTTCACC/ 1	
11501	ACCITICAGE		CGITTATCCC	MCGMXGCA	TUCK NAMAGE	CHICANGER	איככטטטענכ		CCACCCCCAAG	CTISATESCACA	
	TOOMETECE			THACTCGCGT	AGGICTICS	שכעונינינינים	א: א: א: א: א: א: א: א: א: א: א: א: א: א	CCCTCCAGTC	GCTOSCIACTC	GACTACOTAST	
11601	CHARTTABAN		CHENTRACE	GCCCCCATAG	NUNCCUCANG	TCCTACTTRG	ACGCGGGCCC	TGACCTGCGC	TOGGCCCCAA	GCCGACTCGC	
1001	COGACOTTIC			CRCCCACTATC	TECOGETE	ACKINTGANAC	TOCACCCIACO	ACTOGACOCO	ACCCCCCCCTT	cascrococ:	
11701	Cr.markiar.		GACCTGGGCT	GREGGERGEA	בבבניניניניניניניני	CTGGCAAGGT	COCCOCCO	GACCAATATC	ACCAGGGACGA	TCACTACGAG	
	COACCTCCST			CCGCCACCGT	ממוכטנענענוכ	GACCGTTGCA	OCCCCCCAC	CTCCTTATAC	recreetaer	ACTCATCCTC	
11801	CCAGAGGACG		ACCOUNTANTS	TPTCTGATCA	CATCATCCAA			000000000	CTGCAGAGCC	AGCCGTCCC?:	
	OGICHOCTOC		COCTCATGAT TCGCCACTAC	ANAGACTAGT	CTACTACGTT	CHOCHERCE		cocceaceac	GACGICTOGG	TCGCAGC	
11901	CCTTAACTCC	: ACCCACCACT	ageocenon.	CATOGACCIAC				OCCUPACED OC	AGCAGCCGCA	GGCCAMCCFF	
	OCAATTOAGG		TOCCTOCTGA CCOCCGCTCCA	GTACCTGGCG	TAGTACAGUG ACTGACGCGC		GTTAGGM:TO	COCMAGGCCG	TCGTCGGCGT	CONTRACT.	
1000	Control of the Bank	THE PERSON AND ASSESSMENT	Cartector	GCGCGCGCAA	ACCCCACACA CGAGAAGGTG		CTOGCGATCG TANACGCCCT		GCCCGAAAAC	AGGCCATC	
10071					TGGGGTGCGT		GACCIBCTARIC	ATTICCCCA	CCCCCTTTTG	TCCCGGTAG	
	A CONTRACTOR			CHECKE	CCCCTCCCT	CCTTACAACA	GCGGCAACGT	GCNGACCAAC	CTOOLCOOC	TOOLUGGGG	
10171	WOLLLOWICH THE WAY				COCCCACCGA		COCCUMINOCA	concriderrio	GACCTGGCCG	ACCACCCCC"	
,	רמשרות				CACCOCCAAC		GGTTGCACTA	AACGCCTTCC	TGAGTACACA	OCCCOCCAN'	
12201	TOTOCOCOAG						CCAACGTCAT	TTGCOOMGG	ACTCATGTOF	COCCIONATION	
	ACACGCOCTC			יייייייייייייייייייייייייייייייייייייי				ABBUTTERORY	OF ACTION	COCCAGACT	
12301	010000000		_	CTACACCAAC THIGHGAGGG	CACTGCGALT		משייים איזיים פייים יים פייים	TOTAL STATE OF THE	Chromreno	CONTRACTOR OF THE PROPERTY OF	
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			Pst months	_}							
12401	AFTITICCA	A GACCAGTAGA		CAAGRICCTIGC AGACCGTAAA	CCTMARCCAR					CLAICAGGGG	
	TAAAAAAGGT			GENCEGGACG TETGGEATTE	CCACTCGTTC	CHANAGETETE	TOMOGRECE	CCACACACCCC		Control Control	
10201			TRETTEACRE	CANCTICATOR	CTGTTATTCC	TOCTANTAGE		CACACTOCCA	CCOTCTCCC	CGACACATAL.	
10091					GACAACGACG	ACGAITTATCG	CHARAMETER	CTCTCACCGT	CGCACAGGGC	CCTOTOTATE	
	Secretary of the second			Carchenaine	ACCORPANCE	CATALTACATAT	ACTITICCARG	AGATTACAAG	TOTCAGCCGC	いていて」というという	
12601	CTAGGTCACT			SCC A STATE OF	THE STATE OF THE S	CONTRACTOR			ACAGTCGGCG	CCCCACCCCG	
	CATCCAGTGA	A ACCACTGTGA	CATCACCETE	CONTRACTOR	1				Direct		
9	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	the contract of	Charlanne.	TAAACTACCT	CATTERNETANG	HATERICANC CONTRACTOR	AGATCCCCTC	OFFICEACAGT FTAMACAGCG	TTAMCAGCG	ACCACACACAC	
10/71	MOUNTAIN				CCACTIVATING (ACCALCING		TUTACKARAG	CACCTGTCA	ANTIMOTOCOC	TCCTCCT(1)	
10011	Charles	TACCHOCAGE				ANTITIONAL WITTAACOCC	CNACTICATE	CTCCACATGA		CATCGAACTS	
10071	GTAMARGE	GTANACGCG ATGCACGTCG			TACTE Y X TYX	CCCATTRCCG	מתנתומנית מתנידונדותר ממכמכמדו	CACCTIGITACT		STACCTION.	

Figure 15H

PMRKAd5gag MERGR2

GCEATCTTGA CTGTAGAKT ACACHGTGT TGTCGCACAA	CTTGTCCGAT	CTCCTATGC: GACGACCCC TOGACANIAT	ACCTIVITICITY TCTOGOTOTICS AGACTACACC	ATGTTTTAAA	COCCERATORA	CCCCCCTTT GGCCGCCAA.	CTGGTGCACA GACCACCTGT	GREAGGEAAG CCCTCCGTN	CANCINTACC	CCCGAAAACA GGGCTCCCGT	CECHCATCOS CONTRACT	ACCICACATU ACCICACATA TITARGATOA AANTECTARF
TTCACCAAT AAAGTGATTA GACATAGACG CTGTATCTGC	GGCCAAGCAG	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	CTCTCGGATC GTCARGGGGG				CCGTOTGTAC	TACAGCCCGG	TOAACGAGTT	OFTCACGCTG CAAGTGCGAC	CAGACCTT	ANGCETTECH TTCGIANGET CCAGGIAGGGC GGTCCTCCCG
ACCCCTAGTA TYGGACTCAT CT.TCTGACAC GGAGACCTG HINNIII	GCGAAAGGAA AGCTTCCGCA CGCTTTCCTT TCGAAGGCGT	CTCCCACCAC GACCCTGGTG CAACGCGATA	GTTGCCCTAT AGGCACGACC	TTCGCCCCAG	TCCCCTTAGT	CCCTTCGATO	TCGACACCAC ACCTGTGCTG	AAACAATGAC TITGITACIO	ATECCAMATO TACOCITITAC	AGTGGGTGRA TCACCCACCT	ACAGAACGG TGTCTTGCCC	ATATCTTFOC GCFAACCCTT CCCTTCGGAA
GCCGCGTCM T CCGCCGTCM T ACGATGGATTTTTTTTTTTTTTTTTTTTTTTTTTTTTT	CUCTTTCCTT	CTTACCAGCA GAATGGTCGT CATTTCCAA	GTAMAGRETT CCOTCOTCAA GGCAGCAGTT	TTTRCCACACC	TERCTINITAL	CCACCCAAGA	GCACCCLTAT	CCCAGTANGE	CCATACCAAC CGTATGGTTG	CTGAAATACG	AAGTGTGCAG TTCACCCGTC	CONTRACTA CONTRACTA ATCHERAGE TARKENTES
CCATCATA C CCTACATA C CCTGATATA CCTTCACATA	CARTAGATAR ARXIVACERT GGGAAAGGAA ARCTTCCGGA CHCGTTCCTT TCGAAGGCGT TGAAGGCGT TIMINN	TTCCANAITT GATAGESTET AGGETECIAA CTATECECAGA CGAAAAAAAAC CTGCCTCGG	GUCCHTCAC GUCCHTCCAC		GAGCCITTGCT	TRACOCCOCCO	CTCTTAGTTG GCACCCCTAT GAVACTCAAC CCTCGGGATA		AAACCATCCT	Transtagae Agtecaute	CALTACTEGA	CHICTROPORTS CHICTROPOC CANCANCCUG
TYKACTACTT ACCTRIATERA ATTCERVITE TAAGETCEAC		CHERGATECT AGTAGCCCAT TTCCANHITT GATAGGTCT CTTACCAGCA CTCGCACCAC CAGTCTACGA TCATCGCCAT AGGTTCGAA CTATCCCAGA GAATGGTCGT GAGCGTGGTG WATTTCCCAA CAACGGGAAA TCGCTGCTGCTGC GAAAAAAAAC CTGCCTCGGG GATTTCCCAA CAACGGGATA	CCAPATITATES CCAPACICOC CONTRACTOC	TIGGGACTIAG ACCCTCCCTC	CCATTACACC	COCCOCOGIC	CCATCCCTTA	CCACACCAAC	CCCICTGGACT	CTAAGGACAA GATTCCTGTT Pwil		
AACCERTETAA TTKAECERTEE ACACCERTEE	GENNEARTHE	CAGTCTACCT AGTAGCCCAT CAGTCTACG TCATCGGGTA SERVING TCGCTGCTGCTG TCGCTGCTGCTG TCGCTGCTGCTG TCGCTGCTGCTG TCGCTGCTGCTG TCGCTGCTGCTG TCGCTGCTG TCGCTGCTG TCGCTGCTG TCGCTGCTG TCGCTG	AGCGACGACG TCGGCGTCCC CCCAGGAGGA CAGGGACGTG ACGTCCTCAT GTCCCTGCAC		CTCACCAACA	TOTACTCANC ACACCACTCG	CCCTCTTTIGT	ACCAGAACGA	GCACTGGGGC CGTGACCCCG	COCTTOCCTA CTANGGACAA OCGAACGGAT GATTCCTCTTT Pail	TGAACAACGC GATCHTCHAAAAAACAACGCCCCCCCCCCCCCCCCCCCCC	CCCCAANITY CCCMTCACTO CCCCCAANITY CACTOMICAN ACCCACACC CCCTTANCIA TAXATCLUG CGCACTCATT
OCCUTTANTC CRACAMATAG CCTCATTACT GGACCAMAGA	TOCTASAGIT ACGATCISAA			CCACAGCAGC	AAATAAAAAA	CCFACCIACNO GGATGCTCTC	DCO GCCTACCORD	TCCCTCAACT	ACOACCOGAC	GATCCTGTCG	ATAGACCTTA TATCTGGAAT	ACTTCAGACT TGAAGTCTGA GGTGGACTTC CCACCTGAAG
CCTCANACCA GGAGTTTGGC GCTACCGCC CGATGGCGGG	CCGCAGACCC	CTAGGCGCTO CGGCCGCGCGGGATCCGCGGGCCGCGCGCGCGCGCGCGCG	DOATHTOTHO ANDACOTACO	ACTCGGCAGA TGAGCCGTCT	GCATGATGCA	CCTCCTCCCT GGAGGAGGGA Kyrt	E 3	CCTACACCGT	ATCANTCTIO	AGGCGCGCCA	GACCATGACC	
OCCATATATO CCCTACATAC ACCCACACTO TAGGCGTGAC	TTCCCCCCAA	CTAGGCGCTG GATCCGCGAC AGGAGGAGTA	TCCTCCTCAT GAGTAGATGG CTCATCTACC				OTACCTCCOC CACCOACCC		CACACACACC		ACTACTCCGA	
12901	13101	13201	13401	13501	13601	13701	13801	13901	14001	14101	14201	14301

Figure 15I

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CCCCATCETT TOCCATTOEST ACGOTAAGGG GAGAAGCT CTCTTCGTA	COTCHACTOR	GATCACCAGE CACTCCAAGA GTGAGGTTCT Asd	TTTTGCCC(T)	AGTCCAGCON TCAGGTCGCT TTTTGAGCAA AAAACTCTTTT	TOTAL MICHAEL	TTTACTTCT GCACCTATACT CGTGGCCGGC	CCCCBACCATTAN GCCCCCBTTAN CCCTTCATCT CCGTTCATCT TACACATCTA
CTITUTECCOC TRANCOATCA ACTTOCTAGT ACCCOAGOTC TGGGCTCCAG	ACCAGETACE TROOTCATOO	GCCTCGTCA GTTGCCCGTG CAACGGCAC	GAGAACCAGA	CCTAGCCTCC CGTAGCCTCC GAGCCGCACT CTCCGCCTCA	AMGCGCACCC TTCGCGAGGC CCATCGAGGC GGTAGCTGCG GCGCTATGCT	CGCGATACGA CGCGCACGTC GCGCGTGCAG	CCCCCCCCC GCCCCCCCC CCCCCCCCC GCCCCCCCC
					COCOCCANG GCCCCGGTTC GTCGATGACG CAGCTACTGC GCGGAGCCCG		CCATCOCCAR CCCTCCCCAR CCCTCCCCAC CACCCCTC CACCANGCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
	TAAACAATGA	NOCHONCHIA NECTROGNESCO ARENCHICAT TOGNEGECON CEGETHACTIVE GEOCEGNÉCT GRECNECACE EUCOGETECA	ACCTICATIONA TOCACAAGIT		AGARCITIGO TCTACANACC GCGCACCACC CCCCACGTCG		OPECHACACIA CANGECIACI GACGOCIAC ACGARACEAT TACTECOATA
• •		ACCENTINGAC ACCANACGIG CARCANCTIT GREGITGAAA	TCTCTGACCC AGAGACTGGG		ANGOSTICIT GCCGCACTIG GCCGCACTIG CGCGTGACC		ACMOCRETE ACMOCRETE CEMPERCE CEMPERCE CHACKERY A
		TCATAGACCC AGTACCTYGG CGCGCTCAGAT GCGCGGTCTA	CCAGTTTACC GGTCAAATGG	CCFTCTCTCA GGACCAGAGT FTFACAAGAC AAATGFTCCG	CCCGGACGG CCCGGACGG CACAAACGCG GAGTTTGCGC	GRCACCTGCG CCCCTCACCTGCC CCCCTCACCG	TARCAGTRIAC TECTOCACTO TECTOCACTO TECTOCACTO TECTOCACTO TECTOCOCOCO ACCITECT
		COCIANTECOS OCCITAGOSOS TICEGOSECA AAGGCCAGGT	AACTCATOCO	ACTITIOGAA FGCCCCTACG ACCGCGATGC	ACACARSCTG TGTGTCCGAC CTRXGCCGCG GACCCCGCRC		CCGCCCCA GREATIGAG CACATAMICC GREATICATAMICC GREGATICATA
CCATTOTANG CCATTOTANG CCATTOTANG CCATCACCC CCACCCCCCC CCCACCCCCCCCCCCCC		ACCCTCAGAC TGGGAGTCTG CCCCGTGACC GGGGCACTGG	GICTALTCCC	CCACCCTCAG GGRGCCAGTC ACGCCGCACC TGCGGCGTGG	GOSTCOTTAT GOSTCOTTAT ACCGCGCGCG	CACACCACT COTCACCACC GCACCGCTOS	CTCGAGGCT GAGCTTCCGA CAGAGGAAC GTCCCCGTTO GACCCCGTACT CTGAGCATCA
		AACTACOGCO TTGATGCCGC TGATGCAAGA ACTACGTTCT		CCCACCATCA GOGTGGTAGT CTGACGCCAG GACTGCGGTC	CCTTATATCG GGAATATAGC CGCGGGCACT GCGCCCGTGA	ACTACACOCC TGATGTGCGG GCGCGTAGCA CGCGCATCGT	ATGCCCCCC TACGCCCCCCCCCCCCCCCCCCCCCCCCCC
CCTACGATGA TCTGGAGGGT GGATGCTACT AGACCTCCCA AGGGGCAGC AACAGCAGTG TCCGCCGTCG TTGTCGTCAC GGCGACCCT TTGCCACACG CCGCTGTGGA AACGGTGTGC		TECATAC INCOTATO INCOAGNCA INCOAGNCA	OCTICTACIA COACCAGOCC COAGAIGIT GCTOOTCCGG Asci	CCCAGCC CCCCCCC ACCATTA		GACGCCCCA ACTACA CTCCCCCCCO TGATGT GACGGCCCAA GCGCGT CTCCCCCCCCC	ACCOGECTEC TECCCOCCOC AGRECTATGA TCACCATACT TTGCAGGAAA
4501 4601 4701	4801	5001	5101	5201	5401	5601	15801 15901 16001

Figure 15J

16101	ccadotcate	ccapatente acaccadan	TUTATOOLU	CCCDANGAAG		ATTALAARCL	CTCAAACCTA		AAAAGAAAA	GNANGATRIAT	
	COLCCACTAG	COTCCAGTAG COCOCCTCT	AGATACCGGG	AGATACCOOR OCCUTOTIC	בידיר ידונה הרב	キカハヤに・T プに にょ	CCCTTTCCAT	THERECECAGE	THICKLER	CTTTCTACT	
								Sall			
16201	GATGATGAAC	TTGACGACGA	CONTRANCTIC	CTYTCACCCTA		הניהראימבאה הממחרואיםדא	CACTOGAMO	GICGACCCCT	AMARCOTOTT	TIGGGACC:	
	CTACTACTTO	AACTGCTGCT	CCACCTTGAC	GACCITCGAT	CHACOCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOCOC	CRETACCUAT	GICACCTIFIC	CAGCTGCCCA	TITIGCACAA	AACCCTGG::	
16301	GCACCACCGT	AGECTITACO	CCCCGGTCAGC	GCTCCACCCG	CACITACAAG	CACGINITATA	ATCAGGTGTA	CHOCGACTIAN	GACCTOCTTO	ACCAGGCCAA	
	conditional	TCAGANATGC	RECEACTES	CENTICIPATION	GTGGATGTTC	CHARCACATAC	TACTCCACAT	OCCOCTOCTC	CTGGACGAAC	TCCTCCGGFT	
16401	COMPCOCCTC		CCTACGGNAA	-		רהידיבינייביד	CCACGAGTGC	ANCCCANCAC	CTAGCCTAAA	OCCCCTAACA	
	OCTOCOCOLO	CCCCTCAAAC	COATCCCTTT	CCCCGTATTC	CTCTACGACC	GCAACOGCGA	ccrecreces	Troccricio	GATEGGATIT	COCCEATION	
16501	CTGCAGCAGG	PSII CTGCAGCAGG TGCTGCCCGC	OCT TOTACCO	TCCGAAGAAA	AGCGCCCCCT	AAAGTTECCAG	TCTOSTGACT	TOCACCCAC	COTOCAGCTO	ATTATA:CCA	
	GACGICCITCC	ACCIACOCOCCI	CGAACGTGGC	AGGCTTCTTT	TCGCGCCGGJA	THICKRECTE	AGACCACTGA	ACCGROGORO	GCACOTCGAC	TACCATVAGE	
16601	AGCOCCAGCG	ACTOGAAGAT	GTCTTGGAMA	ANATGACCGT	CONNCCTOR	האפשכככם	AGGTECGCOT	GCGGCCAATC	ANGCAGGTOG	COCCGGGACT	
	TCGCGGTCGC	TOACCTICTA	CAGAACCTTT	TTTACTORCA	CCTTCGACCC	GACCTCGGGC	TCCANGCGCA	CCCCGCTTAR	TICGICCACC	GCCCCCTGA	
16701	OCCUTOCAG	ACCOTOGACG	TTCAGATACC	CACTACCALT	AGCACCANTA	THRECARCERE	CACASAGGGC	ATCCACACAC	AMCGICCCC	GGTTGCCTCA	
	CCCGCACOTC	TOCCACCTOC	AGOTCTATOG	CTCATOCITCA	TCGTGGTCAT	AACGGTGGCG	Grencicce	TACCTCTOTO	THYCCAGGGG	CCAACGGAGT	
16801	accortace00	ATCCCCCCCC	CCACCCCGTC	OCTIGOGGCCG	CGTCCAACAC	CTCTACGARG	GTTSCAAACTAG	ACCCGTGGAT	GTTTCGCGTT	TCAGCCCCCC	
	COCCACCOCC	TACOGCOCCA	CONCERCENC	CCACCCCCCC	OCAGGITACTG	GAGATACCTC	CACGTTTACC	TOGGCACCTA	CAAAGCGCAA	ACTCGGGGGG	
16901	000000000	CCCFTCGAGG	MAGTACGGCG	CCGCCAGCGC	GCTACTGCCC	GANTATICCC	TACATOCITIC	CATTICCCCT	ACCCCCGGCT	ATCGTGGCT	
	CCCCGGGCGC	OCCAROCTCC	THEATGCCOC	GOCCOGNICCICG	CCATGACCCC	CTTATACGGG	ATCTAGGAAG	GTAACGCGGA	TOCOCOCCUA	TAGCACCGAT	
17001	CACCTACCOC	CCCAGAAGAC	GAGCAACTAC	CCGACGCCGA	ACCALCACTG	משכנככנכם	CCCACATACGC	CGTCGCCAGC	CCGTGCTGGC	CCCGATTTCC	
	GTGGATGGCG		CTCGTTCATO	GGCTGCGGCT	TGGTGGTGAC	CTTOTOCOGC	ООССОСУОСО	GCAGCGGTCG	OCCACGACCO	GOCCTANAGO	
17101	GTGCGCAGG	TOOCTCGCGA	ACROACICACIO	ACCURAGING	TRICONCAIC	PROCTACCAC	CCCARCATCG	TTTAAAAGCC	CONCINCIO	GTTCTTGCAG	
	CACGCGTCCC		recreecence	TOGGACCACG	ACCIDITION	COCCATGGTO	COCTOCTAGE	AAATTTTCGG	CCAGAAACAC	CAAGAACGTC	
										Sphi	
17201	ATAMOGCCT CACCT	20200	CICCGITICC	COGNACCOGG	ATTCCCGACGA	AGAATGCACC	GTAGRAGGIG CATGGCCGGC		CACOOCCTOA	COCCURCAT	٠
1	TATACCOCCA	GTOGACGCC	GAGGCANAGG		TANCHICTCCT	TCFFACGINGS	CATCCTCCC	GTACCOGCCG	GTGCCGGACT	OCCCCCCCTA	
	155				Sphi						
17301	COMPLETE	CACCACCOGC	300000000	GTCSCACCOT	CTA'ATH ACREG	GCCCTATCCT	GCCCCTCCTT	ATTCCACTGA	TCCCCGCGGC	GATTGGCGCC	
	COCAGCACGC		CCCCCCCCC	-	OCCTARGOUNC	CGCCATAGGA	CCCCCCACCA	TAAGGTGACT	AGCGGCGCCG	CTAACCCCC1;	
17401	GHANDARAA		GGCCTTGCAG	GCGCAGAGAC	ACTGATTAAA	AACAAGTTESC	ATCITICALA	ATCAMATA	ANAGECTOCA	CTCTCACGET	
	Character		CCCCANCETC		TCACTAATT	TRETTCANCO	TACACCTITIT	TASTITUTE	TTTCAGACCT	GAGACTCCTA	
										Econy	
17501	CONTRACTOR	TOTANCTATE	TTCTAGAATG	PHOTAGAATO GAAGACATCA ACTITICOGIC TITICIZICOS	ACTITIOGRAC	היוסאיכנכס	CEACACICACT		CATCCCCAAAC	TRECONGATA	
		ACATTCATAA	MCATCTTAC	CETCTGFMFF	TGAAACGCAG	TISAAACISCAG AGACCITTOLIC	CCTGTGCCGA	GEGEGGGGAA	CTACCCTTTG	ACCGFTCTA1	

Figure 15K

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	Emfly									
17501	Section Action	CARTATGAGE	GETTERCECT	TCACTEROS	دودوروسيه	ACCENTANTA		TTCCACCGIT	NADNACTATO	GCACIC NACTOR.
	AGCCOTOGIC	CITATACTCG	CCACCGCCSSA	ACTUCACTOC	こといいいいいいいい	TYBCCGTAAT	TITTANNOCC .	ANGCTOCCAA	TICITICATAC	CONCONTCO:
17701	CORNE BE BER	Marhraging	AGATTGETTEAG	GGATAAGTTG	AAAGAGACAAA	ATTRICTARCA	AAAACTTGTTA	GATOGCCTGG	CCTCTGGCAT	TAGCGGGGT :
10//1	GACCTTGTCG		TCTACGACTC	CCTATTCAAC	THETET COLLECTIVE	TAMAGGTTGT	TPTCCACCAT	CTACCOGACC	CCAGACCCGTA	ATCGCCCCAI"
1780)	GTGGACCTGG	CCAACCAGGC	ACTGCANAT	ANGATTANGA	GTANY:TTGA	TOCCGGCCF		AGCCTCCACC	COCCETOCAG	ACAGTGTC'F
	CACCINGGACC		TCACGTTTTA	TICTAATIGE	CATTUTUALT	ACKARGERATA	ASSENTATION	TCGGAGGTGG	CCGGCACCTC	TOTCACA
17901	CAGAGGGGCG		CGPCCCCCCCCC	CCCACAGGA	AGAMACTOTA	TETT IN COUNTY	TASACCACIC	recencetae	CAGGAGGCAC	TAUMER AND
	GICTCCCCGC	ACCOCHTITIC	GCAGGCGCGG	GGCTGTCCCT	PCITTICAGAC	CACTAGGTTF	AICTOCTOR	AGGGAGCATG	CICCICCIO	. HOLLES
18001	CCTOCCCACC		TCCCCCCCAT	GCCTACTOGA	ניונא יוייסימכר	איינאייאנאככ	COTARCCCTC	GACCTGCCTC	מנכנננפרופענג	GWZGTCGT:
	CCACGOOTOO	TOGGCAGGT	AGCCCCCCCTA	. CCGATAGGCT	כאכניאכניכניב	Textinonical	פראווארפאר	awaran a	Pag	
	Se secretario	Search Carrey	GACTISTICE	GTTGTAACCC	CICCIAGCCG	CACGITECCTG	כמככמכמכם	CCACCAGACC	OCCUPICATIO	COCCCOTAG
TOTAT	TTTOCACACO		CTOCCOCCA	CAACATTCAGG	CAGGATCAGC	GCCCAGGGAC	ودمعدودهود	GOTCOCCAGO	COCTAGCAAC	GCCCACCATC
10201	TAGINGERA		ACACTGAACA	CCATCCTCCC	Terescent	CAATCCCTVA	AGCCCCALG	ATGCTTCTGA	TAGCTAACGT	GreerArdia
10201	CONCACCON		TOTOACTIOT	COTAGCACCC	AGACCCCCAC	GITTACKGGACT	TCCCCCCTVC	TACCAMGACT	ATCGATTGCA	CARCATACE
18301	TENTOTAT	_	CGCCCCCAGA	ההאתכידיםכיזים	ACCICCICICIO	כמככממכדד	CCANGATAGC	TACCCCTTCO	Afcatocccc	AGREETETT A
	ACAGTACATA	_	GCGGCGGTCT	CCTCGACGAC	Techeology	מכמסטטטאא	CASTITCTACCG	ATGGGGAAGC	TACTACOOCO	ירש באכא
19401	CATACACATE	٤	ACCCTCGGA	GTACCTCACC	CCCCARGCTCG	TECNSTITE	ככפכפככענב	GAGACOTACT	TCAGCCTGAA	TANCAAGTTT
10101	GTACGTOTAG	_	TOCOGAGCCT	CATGGACTCG	CHECECECACE	ACCITCAAACG	ACCACACAGIOG	CTCTOCATOA	AUTCGGACII	ALICI IX MO
19501	AGABACCCCA	g	TACCEACGAC	GTGACCACAG	ACCRETCICA	CECUTACACO	CICCONTICA	recetored	CCCTCACCAT	ACTESCUTAL:
	1011100001		Arccordere	CACTOSTATIC	TOUCCAGAGE	COCAMILLES	GACCCCAAGT	AGGGACACCT	GCACICLIA	Transport
18601	COTACALOGO	g	CTAGCTWTOG	GTGATAACCS	TGTGCTGGAC	ATGGCTTGGA	CGTACTTTGA	CATCCCCGC	CACCACACA	COCACCIA
	GCATOTTCCO	COCCANOTOR	GATCGACACC	CACTATTOCC	ACACGACCTG	TACCGAAGGT	GCATGAGACT	Gracecco	THE CHICAGO	TEBBBTOAN
18701	TITTAAGCCC	TACTOTOGICA	CTCCCTACAA	COCCITORCE	CCCAAGGGTG	CCCCAMITCC	Traccalatica	CATCAGCIC	CINCIPLICATION CONTRACTOR OF THE PROPERTY OF T	ACTITATIVE
	AAAATTCGGG	ATCACACCOT	GACGCARCIT	GCCGCACCTA	CCCTTCCXCAC	CARGILLIAGO	ANCIOCITACO	THE FIGURE	CACCATACT	CCTATAAATA
18801	CTAGAAGAAG	2	CAACGAAGAC	CAACTACACT	ACCANICATION TO THE TANK THE T	COLCOPTIT	TEMETRICE ATA	AACCCGTCCG	CCCRATANGA	CCATATTTAT
	GATCTICTIC		Gricericis		ACCTABATAT	CCCGATAAAA	CATTICANCE	TGAACCTCAA	ATACCACAT	CTCAGTYAGTA
18901	TTACAMAGGA	CONTACTOR	TATTCACACT	-	TRICATTTATA	CCCCTATTIT	GENNAGETICS	ACTIOGAGIT	TATECHETTA	GACTICALICAT
10001	CEANCACAGAN	} E		AGTCCTAAAA	AAGACTACCC	CAATGAAACC	AT : FFACTO	TCATATGCAA	AACCCACAAA	TCANATCC!
	OCHIOCH	42	GTCGACCCTC	TCAGGATTTT	TICTIGATAGG	CHTACTTICG	TACAATGCCA	AGFATACETT	A CONTRACTOR	AATKSCTCAT
19101	COCCARGECA		GCAACAAAT	COANAGETAG	AAAGTCAAGT	CCTTTACGIT	MANGAGET	CARCACTCC	TEGGEORGE	TTACCACTAI
	cccaracca					CCAGACACTC	ATATITICITA	CATGCCCACT	ATTAAAGAAG	GTAACTCACA
19201	ACTTGACTCC TCAACTGAGG	ATTTCACCAT	_	-		GUTCTGTGAG	TATAAAGAAT	CTACCCCTCA	TAATTCCTTC	CATTGNJTG!

Figure 15L

GRIGITCTOG CAGGECANDE ATCONACITY ANTICTITIG TAGATTTOCA AGACANAMO ACAGAGETT CATACCAGET TYTGETTGAT TECATAGENY CEACAAGACE GEEGGRITTEG TAGGETAAC TTACKAAAA ATCTAAAATT TETISTETTIG TGTETGGAA GTATGOTGGA AAACGAACTA AGGTAACGAA

aganctaato ooccarcaat ctatreegaa caggectaat tacattreett tearranta tettategat ctaatgeatt acaacageae oogtaatata TETIGATIAC COGOTOTIA GATAGIZGIT GICCIZINTA ATGIAACGAA ANTXICTGIT AAAATAAGGA GATTACATAA TOTIGICGIG CCCATPATAC

19301

NA.	414	C'A	È		 	TIN: NTM	CT.	TA: ATC	 Þ. D	(C)		TAA VCC	CAA	OTT.	DI LI	たら
AACTICCAAA		ACCTACAGAA	-	-	TOGCTCCCY ACCGAGGGC		GOCCCONGTA	ABTITICATA:	CTTTANCO!			CONCITTAR				TCCAGTAACT
ACTOMAGATO	TGACTICATAC	COCANAMA		CCACATGAGG	ANGCGANTOG TTCCCTCACC	CONTRACTOR CONTRACTOR	CCTTCTCTG GGAAGAGGAAC	OCCACCATTA COCTCCTAAT	ACCACCAGTC TGCTGGTCAG	OCCCCCAAAG		CCCCCAACGA GCCCCTTGCT	ATTICATACTE	CACCTACTAT		CATCCCATTC
NATCATEGA	TITAGTACCT TGACTICITAC			CCTCTTTAAA	CTACATGAAC	AACCACCACC	TTAMANACET CCTTCTCCTG ANTITITGGA GGAAGAGGAC	CCAACTECCT	AACGACACCA TriccTGTGGT	CCCCCAACTO	ACCOACATAT	CCCCTCCTTA	TITACCATC ATTGATATTG	GCACICCAC	TACACACTTC	CCCTTTOOCO CATCCCATTC
CARACTER SANTCATORA ACTORAGATO	CTTAATAACT 1			COGFICCACA	ACACCTACCA (CAACCCATTF 1	TTCTTTGCCA 1	ATCACCTANG TACTOGATTC			-		AAGGACCATG		ACCCCCACC Prof	TYSCENTOCEA
	CENCINICITY OF			CANTETAMAT O	CTATTGGGTT 1		COCAGICTIC A	TCCCTAGGNA A					-		TTGGCTACCT AACCGATGGA	MANTERETT
	CAGCTANTIAT C			CCATCACAAAT C GGTACCTTTA G	AAAAATTTCT G	•	ACATECAACT OF	NGAGC NGAGC			CTCCGGCTAC C		-		TCHOCATTEG 1	
	ACACACATICA C PECCACAACT G		ATCTCTCTO G	MINITING C				TTAACATOGT 1			CATCACTGGG C				ACACAACAAC 1	CAAGACCATA GIIGACAGCA TIACCCAGAA
TAGGGTCAAC T	ATCHCICAATC A		CACACTANTT A	AAGAGTTGGA A						CONCENTRACE A		AGANOGINOC C	GGGTTACAAC CCCCAATGTTG C	ACCTACAAGG A	ACCTACACCA /	PARALCIALA (
	GEACTIFICE A.			AAANTGAAAT A	£ :				-	ATCCCCIACA A CCCCCAACAT G GCCGOTTOTA C		ES	TTGACGGGGA G	TATCCCAGNO A		
		TATCTIONS C	_			OCTAGTOGAC TO			-	GTANACCOM A TATCTCTCC C ATAGAGAGTC G				AGGCTTCTA T		THE PERSON NAMED IN COLUMN NAM
8	19501 A	. T. 19401		19701	19801	19901 0	20001	20101 A	7 20202	20301 7	20401 C	20501 T	20601 A	20701 A	20801 9	

Figure ISM

PMRKAdSgag HERGR2

Hamilia Visanc centecacia certag generect crece cacinecti	45 P 95	AG CCGCCAGESTA AG CGACTCAAGC TC GCTGAGTTCS			CA CTITICANTAN GT GAAAGTIAI" CO CRCATGGCTA THE GROSTAGGGAT		GC GATACACACA GC GTAGCTCAGO GC GTAGCTCAGO	IGG TGACCGTGAC CC ACAGGACGG GC CGTAAGACTT ICG · TTCTGAA	GOCCCCACCO GFTCFTCACG CCGGGGTGGC CAAGAAGTGC
Bank Bondstronaric ercencerage rotacerage Act "YGACGE ACT "YGACGE	AAAOCCATTO TTTCOGTAAC TAGTCAATAC	ATCAGTTATO TTCTGACCAG AAGACTGGTC	TTCAGGTGGG ATCACAACCC TAGTGTTGGG	CAGCITCCTO	ACTACIACA TOATCICTOT OCTICTOCCO		CGCGAGTTGC GCGCTCAACG GCTCCTCCGC CCAGGAGGCG	CATCAAAAGG GTAGTTTTCC AAGAACATGC TTCTTGTAGG	
CATGACTITT GTACTGAAAA ATCGAAACCG TAGCTTTGGC	GCAGGAACTG CGTCCTTGAC GCCTGCGCCA		AACCCTGGACCTT ACTCCCATGG TGAGGGTACC	-		COCCOTCOAG	GOCCTGCGG COGGACGCGC TCCGCGTCCA AGGCGCAGGT	ACCOTACHO TOSCATCACC GCCTTCAGA COGNACIETC	ACCACATTAC TOCKTAAAG
ACCCCTACA TRESCUATET CCCCCCATTE GCCCCCCA	GCTCCAGTGA CGAGGTCACT ACACAAGCTC	TGTGTTCGAG CTCTTTCGAG GAGAAACTCG	ACCOCTGTAT TCCCCACATA CTCCCCCCAA GACCGCCCAA			ACAACCATCC TGTTGGTAGO	TOTOSCETCE ACCCOGAGG CAAGATCAGA CCTCTAGTCT	E S 9 D	CCTCTAGACG
AACTECKGKK FTRAKFKTRIG AGCAKTRIG TRATCGGCGF	GCCCCATOG CGCCATOC THEFTICTIC	AACAAAGAGG AACATGCTAC TTGTACGATG	TCTTCCCCCG AGAAGGGGC CCTTTCCAA GGAAACGGTT	CALICCTOCOF	TOTCACTTGA ACAGTGAACT TINGCGTCTG	AACGCAGAC AAACTCAGGC TTTGAGTCCG	AAGTCCCAGT TTCAGGGTCA COCTCTTGTC GCGAGAAGAG	AGGCTTTGAG TCCGAAACTC AAAGCCACCT TTTCGGTGGA	CGTCCGTGTT
TCTCTACOTC AGAGATECTAT GTCCCATGTAG CAGACACACA	AACAACAGGT TTGTTGTCTA	AAAGCTCCGA CGCACTCAAA GCGTCAGTTT	CCCCATTCT CCCCTAACGA TTTCTCCACG AAAGAGGTGC	AGGTNCAGCC TCCATCTCCG	CACTTCTTT GREANANA ARCCCCACCC	TRACTICCACTT ACTINOCTIGNA Ecoliv	CCATATICTEG CCTATAGGAAC CTTACCAGGA		GREGARCHE CARCANCTTS COTCASTGTT GGAGATTTS CAGCATTTS CAGCACTTS CAGCACTAGAC GCAGCCACAA CCTCTAGACG
RECOMMENT CARTITICIEN CITTISACATO GAMCTICCAC	AAGCAACATC TTCCTTCTAG	TEACHARIACIO ACTOTTCGCG GCCTTGGACC COCACCTTGG	TCCCCCTAG ACCCCGCATC CTCCTCCATG GACGACGTAC	AACAGTCCCC	TTAGGMECCC AATCCTCGCG GTGATTATTT	CACTANTANA TOGRETITAG ACCACAANTE	GCTCOOCCCC CCAGCCCCCC GTCGTCCACG	CCCAAAAGG GGGTTTTTCC TAAAAGGCTT ATTTTCGGAA	
ACAGACCTGG TGTCTGGACC TGTTTGAAGT ACAAACTTCA	ATAMAGAAGE	ACCCATGGAT GATGCCTTT CTACCGSAAA	GAGTCACTCC CTCAGTGAGG GTGGACTATT CACCTGATAA	CTCCATGCTC	AGTOCOCAGA TCACGCGTCT ACACTCTCGG	TOTGAGAGCC GTTGCGATAC CAACGCTATG	GCCATTAGCA CGCAAATCGT TCAGCGCCGG	TAGCTGCCTT ATCCACGGAA AGCGCCTGCA TCCCCGCACCT	TGATTGGCCG GACAGGCGGCACACTACTACGGCGCGCGCGCGCGCGCGCG
GOOCIGCACTIC CCCCCCOTAIAG CTTTANGTTT GAAATACAAA		CCATATATATA GOTATAAAAA GCOTACACTO CCCATGTGAC	GTTTGMGTAC CAAACTCATG TCGGCGGCT AGCCGGGGA	Montacce An CCCATOGOTT	CCCCAGCCAC GOCGICGGTG FTTTAFTTGT	AAAATAAACA GCAGGGACAC COTCCCTGTG	CATCACCAAC GTAGTGGTTG TGGAACACTA	TCAACTTTGG AGTTGAAACC GTTAGGATAC	TGATTGGCCG ACTAACCGGC
PTATOTCCAT O AATACAGGTA O GCCCACCCTT O		ACCAACACC AAGACTGGGG CACATGACCCC	AGOTTIACCA TCCAAATGGT GGGGCCCAAC					OCCARCOCO OCCARCOCO COCTOCOCO OCCAGACOCO	GCCGGAAAAC
21001	21201	21301 21401	21501	21701	21901	22001	22101	22301	22501

Figure ISN

PNRKAdSgay WER682

22601	ATCTICGCCT TO TAGAACCGGA AG	ARCHIGGET TRETAGACTE TAGAACCEA ACGATETEAC	CTRETTEARC	GCCCCCACGC	CCTTTTTCSTT GCAAAAGCGA	CCACTOTACE	ATTTCAATCA	CONCENCENT CONCENT	atttatcata taaatagtat	ATYSCTTCCGT TACGNAGGGA
22701	ACACT	AAGCTCGCCT TTCGAGCGGA	TCGATYTY'AG AGCTAGAGIC	CTGCAGCCGTG GCCTCGCCAC	CNTCCACAAC	CATCHURGE C	TCCCCARTC	ATGCTTCTAG TACGAACATC	GTCACCTCTG	CAANGANTG GITTYICKIN'
22801	CAGGTACGCC	CAGGTACGCC TGCAGGAATC GTCCATGGGAATC	GCCCCATCAT CGTCGTAGTA	CCTCACAAAG	GECTTETTEC	TOTANGOT ACCACITODA	CAGCITOCAAC	CCUCGGHACT	CCTCGTTCAG GGAGCAAGTC	CCAGGICTI-!
22901	CATACOCCO	CATACGGCCG CCAGAGCTTC GTATGCCGGC GGTCTCGAAG	CACTICACTCA GTGAACCAGT Frut	CCCAGTAGTT	TCAAGTTCGC ACTTCAAGCG	CTTTAGATCG	TTATCCACGT AATAGGTGCA	GGTACTTGTC	CATCACCGCG	CISCIACAGE T
23001	CCATCCCTT	CTCCCACGCA	GACACGATCG CTGTGCTAGC	GCACACTCAG	CGCCTTCATK: GCCCAAGTAG	ACCOTANTITE TOGGNETANA	CACTITICCOC	THEOCHOORE AACCOACCO	ADARGCAGAA	CCICTICOTT CCAGAACGCA
23101	CCCCATACCA	COCOCCACTO	CCAGCAGAAG	ATTCACCCCC TANGTCCCCC	recactioned Geotisacaed	GCTTACCTCC	TTTRECEATOR AAACOGTACO	TICATTAGCA	CCOGHGGGTT	CCACTTTC: 17
23201	ACCATTEGTA TOOTAAACAT	GCOCCACATIC CGCGOTGTAG	THCTCTTTCT AAGAGAAAGA	TCCTCCCTCATOT AGGACTGACA	CCACGATTAC GGTGCTAATO	CTCTCTCTCAT	OSCODOCOCT CCCCCCCCCAA	COCCEANCE	AGANGGOCGC	AAGAAAAAGA
23301	TCTTGGGCGC AGAACCCGCG	ANTOCCANA	TCCGCCGCCG AGGCGGCGGC	ACCEPTANTOD TECHNOLITY	CCGCGCCCGAC	CCACACCCCC	GCACCAGGGG	CREARCACTA	GAGICTICCT	CCAOCIA CCT
23401	CTCCATACGC	COCCTCATCC	GCTTTTTTGG CGAAAAAACC	00000000000000000000000000000000000000	CCTCCCCCCCC	GCCACCOGGA	CCCCCTCCTG	ACGTCCTCCA TCCAGGAGGT	ACCAACCCC	ACCAGGGC
23501	OCACCOCOTC COTOOCGCAG	COCCOCTCOCO	CCACCAAAGC	COCTOCTCCT	CTTCCCCACT	CCCCATTACC	TICTIC TATA	CCOTCTTTT	GATCHTGGAG	TCAGTCGM.:A AGTCAGCTC'
23601	AGAAGGACAG		CCCTCTGAGT	TCGCCACCAC	מנוטבוענוטנוטנ מרומנוענוטנוטנו	GATCCCGCCA	ACCCCCCTAC TGCCCCCATG	CACCTTCCCC	CAGCTCCCTO	CCCCCCTTGA
23701	CCTCCTCCTT	OTCATTATCO CACTAATAGC	AGCAGCACCC TCGTCCTGGG	AGGFFFTGTA TCCANACAT	ACCCANGACO TCCCTTCTGC	ACGAGGACCG TCCTCCTGGC	CTCACTACCA	ACAGAGGATA TOTCTCCTAT	AAVAGCANGA TTTTTCGTTCT	CCAGARCAN
23801	OCAGAGGCAA	ACCAGGAACA	AGTCGGGCOG	CCCCTCCTTT	CCCTACCCT	CTACCTAGAT	CACCCTCTOC	ACCHGCTGTT TCCACGACAA	GAAGCATCTG CTTCGTAGAC	CAGCGCCAGT
23901	OCCCCATTAT CCCCCTAATA	. 06	TTGCANGAGC AACGTTCTCG	GCACCCATGT COTCCCTACA	מככככדכמכ במממאמכנים	ATAGCEGATE TATCGCCTAC	TCACCCTTAC AGTCGGAACO	CTACGNACGC	CACCTATTICT	CACCIOCOCOT
24001	ACCCCCAAA	COCCANGAM	ACCICCACATO TOCCOTOTAC	CRACCCAAC	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	ACTTCTACCC TGAAGATGTG	CGTATTTRCC	CACGGICTCC	ACGAACGOTO	GATACTCTA'S Ectely
24101	TTTTCCAAA AAAAAGGTTT	ACTOCAAGAT	ACCCTATCC TOGOGATAOG	TRCCGTGCCA ACGGCACGGT	ACCGCAGCCA	ARCGGACAAG	CAGCTGGCCF	TOCOCCOTCCC	CGCTGTCATA	CCTUNTATO: GCACTATACY

Figure 150

PMRKAdSgag HERGR2

24201	CCTCGCTCAA	CCTCGCTCAA CGAAGTGCCA GGAGCGAGTF GCTTCACGGT	AAAATCTTTG TTTTAGAAAC	ARGENT TING TCCCAGAANC	ACGCCIACIAG TGCGCTGCTC	AAGTGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGGG	CAAACCCTCT GTTTGCCAGA	CCANCAGGAA CCATTGTCCTT	AACAGCGNAA TYGTCGCTTT	ATGNAAGTCA TACTITCAGT
24301	CICIODAGIO	THUSTICIANC	2	CAACGAGATA	CTAGCCGTAC	TANAACTICAG	CATCEARRATC	ACCCACTITO TOXGTGAAAC	CCTACCCACC	ACTTAACCTA TGAATTGGAT
24401	CCCCCCAAGO				TV:CCTCCTCC ACCXTGGGCAGG		GAGAGGGATG	CAAATTIGCA	AGAACAAACA TCTTGTTTGT	GAGGAGGGC" :
24501	TACCCCCAGT	TOCCONCOM ACCOCTOCTC	CAGCTAGICG	GCTGACTTCA	AACGCCGCGAG TTXCCGCCTC		TREATHERAGED ACCTCC/TCGC	ACGCAAACTA TGCGTTTGAT	ATCATOGCCG TACTACCOGC	CAGT :CTCGT GTCACGAGGA
24601	TACCGTGGAG ATGGCACCTC	CTTGAGTGC	Sp## .A TGCAGCGGTT ST ACGTCGCCAA	CTTTGCTGAC	CCGGAGATGC	AGCGTAGGT	AGACCANANCA Tepectitist	TECACTACA	CCTITICANCA	GGGCTACGTA
24701	COCCAGGCCT	88	CAACOTOGAO	CTCTGCAACC	TGGTCTCCTA ACCNGNGGAT	CCPROBATT	TTGCACGAAA	ACCOCCTTOO TOOCOGAACC	COTTTTGCAC	CTICATTCCA
24801	CGCTCAAGGG GCGAGTTCCC	CCAGGCCCCC	3 2 8	TCCGCGACTG AGGCGCTGAC	CGTTTACTTA	TTICTATGCT	ACACCTGGCA TYTCGACCGT	GACGGCCATG CTGCCGGTAC	OCCOPITIONS CCOCMACCO	AGCAOTISCTT TCGTCACGAA
24901	GCAGGAGTGC	AACCTCAAGG	AGCTGCAGAA	ACTGCTAAAG TGACCATTTC	CANAACTIGA	AGGACCTATG TCCTGGATAC	CHOCCOGANG	AACGAGCCT	CCCHOCCOCC	GCACCTGGC11 CGTGGACCGC
25001	GACATCATTT		CCTGCTTAAA	ACCCTRICAAC TRAGACGTTG	MAKETETOCC TCCCAGACGG	AGACTICACC TCTGAAGTGG GTGCCCATTA	ACTACCCCCA ACTACCCCCAA	TOTTGCAGAA ACAACGTCTT ATGCCCTCCG	CCGCTTTGGG	TTTATCCTW' AAATAGGATY' GCCACTGCTA
25101	TOCOGRATICO	TTAGAACGG		CACGTGAAGG	ATCCCTGAAA		TCATGGCGCT	TACOGGAGGC	GCCGAAACCC	COGTGACGAT
25201	CCTTCTGCAG GGAAGACGTC	CCTTCTGCAG CTAGCCAACT GGAAGACGTC GATCGGTTGA	ACCPTOCCTA TOGAACCGAT	CCACTCTGAC	ataatggaag tattaccttc		COSTCI'A OCCAGAT			
25301	ACCCCGCACC STGGGGGGTGG	GCTCCCTGGF CGAGGGACCA	TOCAATICO		ACCANAGICA		ANTTATCAST ACCTITICAGE TTAATAGECA TOGAAACTEG			GANAGICCO CTTTCAGO
25401	CONCRETE	CAACTTICAG	ACTECCIODOC TGAGGECECE	TOTOGACGTC ACACCTGCAG	CCGNATGGNA		TACCTGAGGA	CTACCACGC	GTGCTCTAAT	CCAAGATISCT
25501	AGACCANTCC TCTGGTTAGG	COCCOCCTA	ATOCONAGET TACCCCTCGA		-		ACANCCOGIT	AACGTTCGGT	AGTIGITICS	ממכמידוכיוי
25601	AAGACGATG	GANAGOGACO CETTICCCTGC	CCCCCAAATO	TTOSACTICEC AMILITAGAGG	ACTOCOCOCT TCACCCCCT	CCTCGAGTTG	CCAMICCCC	GCCCCCCCCCCCT	CCCCATAGIC	Greenedon

Figure 15P

phrkadigaag nergra

125

				-	3					
25701	3000000000000000000000000000000000000	TICCCAGGAT	CCCACCTANA	AAGAAGCTGC	AAGAAGCTRIC ALK TREETER	נייבענינעיפ	CACGAGGAAG	MINCHOODA	CAMPCAGGCA	CARRAGATIFF
•	CCCOOGAACG	AAGGGTCCTA	CCCROASIFIE	Tretregand	Try: Arxidican	CINTRACTAGE	CINCINCIPLE	TTATGACCCT	OTCACTCCGT	CHECHICAN .
						tmd[[]				
25801	TOCACGACCA	CCACCACCAC	ATCATCAAG	ACTORGRAPH	נינידאהאהימאה	מאאהכידיבכה	AGGTCGAAGA	GETGTCACAC	GAAACACCGT	CACCTICGY .
	ACCTGCTCCT	CCTCCTCCTO	TACTACCTTC	TGACCCTCTC	CONTICTOLITY:	CITCGAAGOC	TCCAGCTTCT	CCACAGACTO	CTTTGTGGCA	GTGGCAGCC1.A
25901	CGCATTCCCC	Traccadacac	CCCACANATC	GGCAACCGGF	TCCACCATGG	CTACAACCTC	CACTCCTCAG	OCCCOCCOC	CACTGCCCGT	TCPCCCACCC
	GCCTAAGCCC	AGCGGCCGCG	CONTENTAG	CCCTTRACCCA	ACCITICATACE	CATHITTICAL	CCGAGGAGTC	crecesses	GTCACCOCCA	ACCOCTGGG
26001	AACCGTAGAT	GOGACACCAC	TOGANCCAGG	OCCUGITANGE	CCMACAGCC	CCCCCCCTTA	CCCCAAGAGC	AACAACAGCG	CCAAGGCTAC	COCICATEO"
	TTOOCATCTA	ceeteteens	ACCTROGACC	CCCCATTCA	GSTTCOTCOS	CRECARCIAT	COGETICACO	THGTHGHCGC	GGTTCCGATG	OCCINGTACCO
26101	GCGGGCACAA	GAACGCCATA	ornecticer	TOCANGACTO	TUCCHCICANC	ATCTCCTTCG	CCCGCCACTT	TETTETETAC	CATCACORCO	TEXXX THUSE
	COCCONOTT	CITICOCOTAT	Chacchacga	ACCITICTION	ACCECEGITE	TAGAGGAAGC	GCCCCCCCAA	AGAMGAGATG	GTAGTGCCGC	ACCTICAACGT
26201	CCOTARCATC	CTCCATTACT	ACCONCATCT	CTACAGCCCA	TACTRICACER	GCCACAGCCG	CAGCAACAGC	ACCOCCACA	CAGAAGCANA	GREGACEGGA
	OCCATICITAG	GACGTANTGA	TCCCAGTAGA	CATGICOGGI	ATGACGTYGC	CACCGTCGCC	Gregingica	receeserer	Grenteam	CCGCTGGCCT
26301	TAGCAAGACT	CTGACAAAGC	CCAAGAAATC	CACAGCGCG	GCARCAGCAG	GADGAGGAGC	ocrocorcia	GCGCCCAACO	AACCCGTATC	CACCCGCGAG
	ARCOTICTOA	GACTOTITICS	GGTTCTTTAG	GTGTCGCCCC	COTECTICATE	CTCCTCCTCG	CGACCCAGAC	COCOCCTICC	TTGGGCATAG	CTGGGCGCTC
26401	CTTAGAAACA	GCATTETITICS	CACTOTOTAS	CCTATATIFIC	AACAGAGTAG	GROCCANGAA	CANGACCTOA	AAATAAAAA	CACCICICIO	CCATCCCTCA
	GAATCTFIGE	CCTAMMAGG	GTGAGACATA	CCATATAAAG	TRESCRICTOR	CCCGGTTCTT	GITCICOACT	TITATIFIE	GTCCAGAGAC	OCTAGOGAGE
26501	CCCOCAGCTO	CCTOTATCAC	ANACCGAAG	ATCAGCTTCG	GCGCACGCTG	האהאהמכפס	AGCTOTOTA	CACTALATAC	TOCCOCOCTUA	CTCTTAAGGA
	GOCCOTCGAC	GCACATAGTO	frincocinc	TACTCCAAGC	CCCCTRACTAC	CFFCFGCGCC	TCCGAGAGAA	GICATITIATO	ACCCCCCACT	CADAATTCC .
26601	CTAOTTTCOC	OCCUPACIO	ANTITARGE	GCGAAAACTA	CGTCATCTCC	AGCOCCACA	CCCCCCCCCA	OCACCTOTTO	TCAGCCCCAT	TATITAGENAS
	GATCAAAGCG	COCCANACAC	TITAATICO	COCTETTICAT	CCACTAGAGG	TOCOCOGIGE	OGOCCOCOOT	CCTCGACAAC	AGregogota	ATACTCOTTC
26701	GANATICCCA	COCCCTACAT	GTGGAGTTAC	CARCCACAAA	TGGGACTTGG	OCCTGGAGCT	CCCCAACACT	ACTICAACCCG	AATAAACTAC	ATGARCCCC
	CTITARGGGT	OCCOUNTERA	CACCTCAATG	Greenerr	ACCCTGAACG	CCGACCTCGA	COCCUTCTGA	TCACTTGGGC	Tratificato	TACTCGCGCC
		Ecofiv			₩ ¥	Econi				
26801	GACCCCACAT	GATATCCCGG	GTCAACGGAA	TACOCOCCCA		ATTCTCCTCG		TATTACCACC	ACACCTCOTA	ATAACCITAA
	CTOCOCATGTA	CTATAGGGCC	CAGITOCCIT	ATGCGCGGGT	CARCIFICACT	TANGAGGACC			TUTCAMGCAT	TATTCCAATT
26901	receestags	TOCCCOCTO	cccraststa	CCAGGANAGT	CCCCCCCCCA	CCACTGTGGT	_		CCGAAGTTCA	CATCHANC
	AGGGGCATCA	ACCOMOCIGAC	GGGACCACAT	GGTCCTTTCA	GAGCGAGGGT	GGTGACACCA	TOMOGRACT	CICCOCCICC	GCCTTCAAGT	CTACTGATTG
27001	TCAGGGGCGC	AGCTTGCGGG	COCCUTICGE	CACARGGING	ממורהכככהה	CCAGGGTATA	ACTICACCTGA	CANTCAGAGG	OCCAGCTATT	CARCTCAACT
	AGTOCCOCOCO	TEGNACOCC	GCCGNANGCA	OTGTCCCACG	CCANCAGACC	CCTCCCATAT	TOAGTOGACT	GTTAGTCTCC	CCCTCCATAA	Greanstra.
27101	ACCASTCOST	GAGCTCCTCO	CFFICCFICTICC	GTCCGGACCEG	GACATTICAG	ATCGGCGGC	-		CCTCGTCAGG	CANTCCTAAC
	TOCTCAGCCA	CTCGAGGAGC	GAACCAGAGG	CAGGCCTGCC	CTCTAAAGTC	TAGCCACCAC	OCCCOCCOAO 1	AAGTAAGTGC	GCAGCAGTCC	GITACCATTC
	Pati									
27201		recreeking	ACCCCCCCC	TOGAGOCATT	GCAACTCTGC		CCTC PARCEC	CCATCOGICT A	ACTITION TO THE	CHICKGGA
	AGACGECTOG	AGCAGGAGAC	TCGCCGCGAG	ACCITCOTA	Ct. Trincht.	Timorina				

· Figure 1561

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00 4. 00	TANTALATO AGNATAN.A ATTATTATO TCTTTAAT' TACTTTAAC ATCTCTCO ATGAAAATTO TACAGGGS'A	AACKCCACC TCCTTACCT*1 TTOTOGTOGO ACGNATGIAC TCATAACTC TGTTTACCAG AGTTATTGA ACANATGGTC		ANGGEROSCE GOCTOCINE TECCGAGGG COGACGACAC TCACCCTTGC GTCAGCCCAC AGTGGGAACG CAGTCGGGT11	ATATANTOC ACCACAGN ATATTTACO TOOTOTCT1:} GAGTATAATO TTACAGTT1 F CTCATATTAC AATOTCAAM	AGANAMOTT GFOSCCCCA TCATATTCAA CACCGGGAGT CTATATTAA TACANAGCA. GATATAATTT ATGTTTTC '' CTCCTTGCAA AACANATT''A GACGAACGTT FTGTTTAU 'T TCTATGTGG ATATGCTC'A AGATACACC TATACCAGGT
ACTOAATOTT TIACTTACAA CTTTGAATTO GAAACTTAAC CGCCCCTGC	GTOCTEMOTA CACGACTCAT CCTTACCTOG GGAATCGACC	CATCAGAAAA GTAGTCTTTT CCGACAGACC GCCTGTCTOG	AATTCAAGCA	TTCTCTGCCT AAGAGACGGA CTAGGTTTAC GATCCAAATG	GCACCACTCT CGTGGTGAGA TGACACTACA ACTGTGATGT	ATGAGCAMC TACTCGTTTG GTACCCTACT CATGOGATGA GCTTTACTCG CGAAATGACC AACAATTGAC TTGTTAACTG
GGACTECACC CHARTATIC CETCACCCTC CTAINTAIG GACTIVEGGTG ACTITIVACTA CTAACCCCC TEANANGAT TEATTECAGG GTTTACCCAG ACTAACCCCT CAANTGGGTC	AGATETTTGT TOCCATETET TETAGAANCA ACGETAGAGA CETAGGAAA CEANFRIGAA GOGFFEGTTT GETTEGGETT	CTCTCCGNOC TCACCTACTO GAGAGGCTCG AGTCGATGAG ACCGTANACC AGACTTTTTC TGGCATTTOG TCTGAAAAAG		CTTTATTCTT ATACTACCE GAANTAGAA TATGATTCCG AGATTAG GTACATAATC TCTACTAATC CATGINTING	COCAGCTGAA OCTAATTAOT GCATCGACTT CGATTACTCA TATGCTATTT (RCAGCCAGG ATACGATAAA CCOTCGGTCC	ACACCCTCTA TACCATGTAC ACACCCTCTA ATGGTACATG TACAGTGCTC GCTTTTTGTCT ATGTCACGAG GGAACCAGA ACTAATGTC ACCACTAACT TCGATTACA TGGTGATGA TACTCAATAC ACGAGTTATG GTAAGGGGAC
ACGCGTATTANA TOCHTACCCG GALVATTACCG GACCCTACAC CCGGCCATCGG	CHANTSTAGT CHANTSTAGT TETTEACCCG ASAASTGGCC	ACCIDITATION TECTICATED CTACCOCCTG	MARGCECKG	THATGATTCT AACACTANGA TCGCCACCCA AGCGCTGCGT	ATGTTACATT TACAATGTAA GTATCATGTT CATACGACAA	TITIATGAMA ANANTACETT CTANGCTAAT GANACGATTA TANGTTACAA ATTCAATGT CCTCATTTCC
TCAATITATT CCTAACTITG AGTTAAATA GGATTGAAAC CACTGTCCCC GCCACAACTG GTGACAGCGC CGGTGTTCAC TTACGCCCA GGGAGACTT AATGGGGGT CCCTCGAA	AACGTTCACTOT CCTAACCCTG AACGTTCACA GGATTGGGAC CCATCCTGTA AACGCCGGGGGGGGGGGGGGGGGGGGGGG			GOTHGGGHT ATTCTCTGTC CCACCCCAA TAAGAGACAG CAGCTTTTTA AACGCTGGGG GTCGAAAAT TTGCGACCCC	TTTTANGGNG CCAGCCTGTA AAAATTCCTC GGTCGGACAT CACAAANCA AAATTGGCAA GTGTTTTTGT TTTANCCGTF	CHTTRICTA TACTITICCA GAAAATACAT ATGAAAAGT AGCGGAAAG ACGACGTGA AACAAAATGC CTTAATTTAC TTCTTTACG GAATTAAATG GAATAGGATT TAAACCCCCC CATATCCTAA ATTTGGGGGG
ACTATCCOSA TGATAGGCCT ACACCTOSTC TGGGACCAG GGCGTCCOGC	TCACTOTGAT AGTGACACTA GCTCCTATCG	CAACMSTATC GTTGTCAAAG ACGAGTGCGT	GAGCTFAGAA CYCGAAICTT Xbs1	MCGC MTGT	AAAAGGTUGA TTTTCCACCT GCTTATTCGC CUAATAAGCG	ACTEATATA TEAGRAMENE ACETITION TATIGAGGA ATACTECTT ATTATAMITA TAATATAMIT
1 ccreccooce gologocicos 1 tococctola Acocoolecti 1 cccoocecae golococoto	CCCTGTGTTC GGGACACAG AAATACTGG		AACAGGAGG	TCAGGTTPC AGTCCAAAG TGCACATTI ACGTGTAAA	North Control of Contr	01 CCAGGGFAMA GGFCCCAFFT 01 CAAAATGGFG GTFTFAWGC 01 GACGCAGCTF CFGCGFCGAA 01 AAAAGTFAGC TTFTCAAFCG
27301 27401 27501	27601	27801	28001	28101	28301	28501 28601 28701 28801

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28901	GCGCTACAAC	CTTGAAGTCA			ATCTCARCAT CTCACTTTCS	CCAGCACCTG	TCCCCCCCGAT	THUTTOCAGE	CCAACTACAG CGACCCACTC	CCACCCACTC	
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29201	TATACTOR	_	ACATTCAAAC	AATCATCAA	Treatheat	SENTENCES OF STREET		T. P. P. P. C. R. P.	TETTIFICATE TACAGTATGA	TTAAATGAGA	
	ATATCAGGGT		TOTOGGTTTO	TTACTACT	ACCTATCTAA	CCTGCCTGAC			ATGTCATACT	AATITACICT	
		Xhoi									
29301	CATCATTCCT CCAOTT	SCT CCAGTITITA	TATTACTORC		CETTGITIGED CITITITIONS CONSCIECAC	CONSCIECAC	ATTOOCTOCO	GFFICTCACA		CTCCATTC A	
	. GTACTAAGGA	CTACTARGGA GCTCAAAAT	ATAATGACTG	GCAACAACGC	GAMMANACAC	GAAAAAACAC GCACGAGGTIG	TAACCGACGC	CAAAGAGTGT	AGCTHCATCT	GACOTANO.T.	
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29401	GCCTTCACAG	TCTATTHGCT	TTACGGATT	GTCACCCTCA	כניכובעו	CAGCCTCATC	ACTIONOGREA	TCCCCTTTAT	ACTIVITACITEA TECCEPTITAT CEAGNICEATT GACTGGGTT.	GACTOGGTCT	
į	COGAROTOTC	ACATAAACGA	AATOCCTAM	CACTGCCAGT	GCGAGTAGAC	GCGAGTAGAC GTCGGAGTAG	TGACACCAGT AGCGGAAATA	AGCCOONATA	GCTCACGTRA	CTGACCEANA	
							EcoFI	-			
29501	STOTOCOCTT	TOCATATOTO	AGACACCATC	CCCAGTACAG	GGACAGGACT	ATAGCTGAGC	THOTTAGANT	TICTTAGAST TOTTTASTTA		TOTONOTH"	
	CACACGCGAA				CCTGTCCTGA	TATEGACTEG	ANGMATCTTA AGAMATTAAT	AGAMATTAAT	ACTITABANO	ACACTICANA.	
29601	CTCCTCATTA		ATCTGCGTTT	TOTTCCCCGA	CCTCCAAGCC	TCAAAGACAT	ATATCATICA GATTICACTEG		TATATEGAAT ATTECAAGIT	ATTCCAAG11	
	GACGACTAAT			ACANGGGGCT	CCACCTTCCC	MOTTECTOTA	TATAGTACGT	CTAAGTGAGC	ATATACCITA	TAAGGITCAA	
			•				Pstl				
29701	CCTACAATGA	AAAAAGCGAT	CTTTCCGAAG	CCTCASTTATA	TCCAATCATC	TCTCTTATOG	TOTTICTOCAD TACCATCTTA		OCCUPACTA TATATCCC . A	TATATCCCIA	
	CGATGTTACT			GCACCAATAT	ACGITAGIAG	AGACAATACC	ACAAGACGTC ATGGTAGAAT		COOGNICOAT ATATACKCAT	ATATAGGAT	
29801	CCTTGACATT	OCCTOGNACE	CANTRIATICE	CATGAACCAC	CCAACTITICC	כבפכשבבנופב	TATGCTTCCA	CTCCACAG	TIGITISCOS COSCITIVATO	COCCUTICAL	
	CONCIGINA		GTTATCTACG	GTACTTGGTG	GCTTCAAACG	COCCCCCCCC	ATACGAAGGT	GACGITIOTIC	AACMACGGCC GCCCANAILA	SCCCANALY I	
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29901	CCAGCCANTC	Agetheace	ACCTICACCC	ACCUCCACTO			ACAGGAGAGAG. ATGACTGACA			AGAMATCAC	
	OCTOCOTTAG	TCGGAGCGG	TOGANGAGG	TRACOCOLICAC	TITAGICIONE	GANATTAGAT		TACTOACTOR		זרזוושררוט	
10001	GCAATTAITA	CAGAGCAGCG	CCTGCTAGAA	AGACGCAGGG	CAGCGGCCGA	CCAACAGCGC				THECACCAGE	
	CCTTAATAAT		GCACGATCTT	TCTOCOTCCC	GTCGCCGGCT	COTTGTCCCG	TACTTAGTTC	TCGAGGTTCT	CTACCAATTG	AACCHOOPEA	
10101	CECANANGGG		CTCGTAAAGC	ACCCANGE	CACCTACGAC	AGTAATACCA				CCANGCGT""	
	CONTINUECCC	ATAGAN	GACCATTICG	TCCOGTTTCA	CTCCATCCTC	TCATTARKET	GCCTGTGGC			GOTTH: CF AL:T	
10201	CANATICACIFIC	CAAATTGCTG CTCATGOTGG	GAGANAAAGCC	CATTACCATA	ACTCAGCACT	CRETARAMIC	CGAAGGCTGC			ACCTGARGAT	
	CTTTARCCAC	CAOTACCACC	CICITITICGG	GTAATGCTAT	TGACTCCTGA	OCCATICITITIE	GCTTCCGACG	TANGTGAGTG	GAACAGTTCC	TOCACTOCTA	
				Brill	1						
30301	CTCTGCACCC	CICTOCACCC TEATTANGAC CCTGTGCGGGT CTCAAAGATT.	CCTOTOCOOT	CTCANAGATE	TTATTCCCTT	TAACTMATAA AAAAAATAA TAAAGCATCA CITACTTAAA ATCAGTTAAC	MARKANTAR	TRANSCATCA	CTTACTTAAA	ATCAGITAGE	
	CACACOTICCO	GACACOTOGO AATNATICTO OGACACGCCA	GGACACGCCA	GAGTTTCTAG	GAGTITICTAS ANTANGGRAN ATTICATIATT	ATTCATTATT	TITITITITY ATTRECTAGE GAATGAATT TRITCAATU	ATTTCGTAGT	GAATGAATIT	TRITCARICO	

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30401	AAATTTCTGT	CCAGTITATI	CAGCAGCACC	nectronect	CCTCCCACACT	CHATTATTEC	AGCTTCCTCC	TOCCHOCAA	CTTTCTCCAC	ANTCTABATO PTAGATTTAC
10501	CA STUTE BUT	THE CHAPTER	Techarecar	CCGCACCCAC	TATETTE	TTETTTETAGE	TGAAGCGCGC	AAGACCOTOF	GAAGATACCT	TCAACCCC(:T
	CITACAGICA	AAGGAGGACA	ACCACAGGTA	CARRETTO	ATAGAAGTIAC	ACACCTCT	ACTICAGGG	TTCTCGCAGA	CFICTATODA	ACTICOCC A
30601		GACACCOONA	cceatectec	MCTGTGCCT	THETTACTE	CHCCCTTIGT	ATCCCCAAT	CCCTFTCAAG	AGAOTICCCCC Tr Tr AGGGGG	TOGGGTACT:
	CATAGGTATA	CHEROCCETTE	GECCAGGAGG	THEACACGGA	AMGANTGAG	CACCOMONICA	X1.0555VI			
				or comments	******					
30701	TCTTTGCGCC	TATCCGACC	TCTAGITACC	TCCANTIOCCA	THURDOCT	CAMMATCAGC	ANCHOLOGIC	CICTOGACGA	פירנייניושר	בנושררורי.
	AGAMCGCGG	ATACCCTTCC	AGATCANTGG	ACCTTACCGT	ACCIMICOCUA	GETTTACCCG	TTOCCOCIONA	GAGACCTGCT	CCGGCCGFFG	GANTGCAG()
30801	AAAATOTAAC	CACTGTGAGC	CCACCTCTCA	AAAAAACCAA	GTCMMCATA	AACCTOGAAA	TATCTVALACC	CCTCACAGTT	ACCTCAGAAG	CCCTAACTOT
	TITTACATTO	GROACACTCG	COTOCAGAGE	TITTINGGIL	CAGTTTGTAT	TRKINCCTT	ATAGACCITOS	CCAGTGTCAA	TOGAGICTIC	GGCATIGACA
10901	COCTOCCOCC	GCACCTCTAA	10c1ccccc	CAACACACTC	ACCATCICAAT	CACAROCCCC	CCTAACCGTG	CACGACTCCA	AACTTAGGAT	TOCCACCCAA
	CCGACGGCGG	CCTCCAGATT	ACCAGCOCCC	GITCICICAG	TECTACGITA	GTGTCCGGGG	CCATTCCCAC	GTGCTGAGGT	TIGAATCOTA	Accordage !
31001	OGACCCCTCA	CAGTGTCAGA	AGGAAAGCTA	OCCCTGCAAA	CATCAGGCCC	CCTCACCACC	ACCGATAGCA	GTACCCTTAC	TATCACTOCC	TCACCCCCTT
	CCTOCOCOACT	GTCACAGTCT	TCC3TFTCGAT	CCCCACCTTT	GTACTCCRCG	CCACTOCTOC	TOCCTATEGE	CATCCCANTG	ATAGTGACGG	AGTCCCCCAA
31101	TAACTACTOC	CACTGGTAGC	THOOCCATTG	ACTTGAAAGA	OCCCATITAL	ACACANANTO	GANAACTAOG	ACTARAGING	OCCOUNT	FOCATIOTAL .
)))	ATTOATORCO	GTGACCATCG	NACCCGT/NAC	TGANCTITCE	COCGINAATA	TOTOTITIVE	CTTTTGATCC	TOATTTCATO	CCCCANOCAN	ACCTACATA
11201	ACACCACCTA	AACACTITICA	CCGTAGCAAC	TOGTCCAGGT	GREACTATTA	ATAATACTTC	CTTGCMACT	ANGITACTO	GAGCCTTOCO	TITICATICA
	TCTOCTODAT	TICTCAAACT	OCCATCGTTG	ACCAGGTCCA	CACTCATAAT	TATTATCAAG	GAACCITICA	TTTCARTOAC	CICODAACCC	NAACTAAL .
11101	CARCTCAATA	TECARCTERA	TURACCAGGA	GCACTAARGA	TRIATTICTCA	NACAGACGC	CTTATACTTO	ATOTTACTTA	recontribat	GCTCAAAA CC
	Griccorrat	<	ACATCGTCCT	CCTGATTCCT	AACTAAGAGT	rmoretece	GAATATGAAC	TACAATCAAT	ACCENANCTA	CCAOTITION
11401	Barra Barra		CACACCCTC	TITITATANA	CTCAGCCCAC	AACTTOCATA	TTAACTACAA	CAMOOCCITY	TACTIONITY	CAGCTTCAN
10216	THEATTTAGA		GICCEGGGAG	ANANATATIT	GAGTEGGGTG	TTGAACCTAT	ANTTGATGIT	GTTTCCCGAA	ATGAACAAAT	GICGAAGITI
		Handiii								
21601	A B statement & A D	A P. C. P. T. P. C. P. P. C. P. P. C. P. P. C. P. P. C. P. P. C. P. P. C. P. P	TTAALTTAAG	CACTGCCANG	GOSTICATOR	TTGACGCTAC	AGCCATAGCC	ATTAATGCAG	GAGATOGGCT	TOWILL !
70070	THAGGITT	TTCOMCTCC	AATTOGATTC	GTCACGGTTC	CCCNACTACA	AACTOCGATG	TOGGTATOOS	TAATTACGTC	CICTACCCGA	ACTIVAACCA
31601	TCACCTAATO	ט	AAATCCCCTC	AAAACINAAA	TRACCATOR	CCTAGAATTF	GATTCAAACA	AGCCTATOGE	TCCTAAACTA	COAACTCACT
	ACTOCOATTAC	STOCTT TOTO	TITACCCCAG	THICHT	AACCGGTACC	GGATC TTAM	CTAAGTTTGT	TCCGATACCA	ACCATTICAT	בכבומשררומי
11701	Tradition C	CACCACACAGOT	OCCATTACAG	TAGGNAACAA	ANTANTOAT	MOCTAACTT	TYTERACCAC	ACCAGCTCCA	TCTCCTAACT	GTAGACTAAA
	AATCANAACT	GREGIOTECA	COGTANTOTC	ATCCTTTGTT	TTTATTACTÀ	TTCCATTGAA	ACACCTRACTG	TOCICGAGGT	ACACCATTICA	CATCTGATTE
11801	TOTACACAAA	C	TCACTITIOGS	CTTAACAAA	TUTAXACTO	AAATACTTGC	TACAGITICA	CFFFFORCTO	TTAMAGGCAG	THOGGMCCA
	ACONCICIA	ACOTOTOTOT CTACGATITO	AGTGAAACCA	GAATICITIT	ACACCCTICAG	TTTATGAACG	ATCTCANNOT	CAAAACCGAC	Armeene	MACCCAGGT
1.001	A De Later Street	PERSONAL CAMPUSANG	TOTAL	ATTATAGAT	TTCACCAAAA	TEXCAGTGCTA	CTANACAATT	CCFFCCFGGA	CCCAGAATAT	A. AACTTA
70616	TATAGACCTE	TATAGACCIT CICAAGITIC	ACCAGINGAA	TAATATTETA	AACTGCTTTT	ACCTCACGAT	CATTICITAA	GGAAGGACCT	GOCTUTATA	ACCTICAAAT
	Bild									
10001	T ADMITTAGE T	TETTACTON	OCCALANCET	ATACAMACCE	TGFTCGAFTF	ATTICCTANCE	TATCACCTTA	TCCANAATCT	CACCCTANAN	CTGCCANAG
, ,	CTTACCTCT	CTTTACCTCT AGNATGACTT	CCGTCTCCGA	TATGETTECC	ACAMCCTAAA	TACGGATTOG	ATAGTCGAAT	ACCITITINGA	GTGCCATTIT	GACGGGTTTTC

Figure 15T

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ATCTCCCOAG CCACACTUAT TOCTANAMA TIGITOCCAN CHINATICATING ACANANATA TCANGGEAMA AGGETGENAT GENOTIGAAGG GCTAGGGCAT AACAACCCTT COTCCCATC AATAGCACCC TTATCGROOM CACTORIACA GTCACCTOTO CTTATTCGCT GTGGGTCGAT ANCCTI 'NN CACANTOGCT ACCATOCCCA TOCTACOOCT CCTCCACCTT GCAGCTGGAA CGATCCCGTA **GGTGTGACTA** TACAGACAT CTOAGAGACT TITICGAGITI CTCTTPACCC! PCCFFCGFAC TGFFFFCFFG GCCTAGGCAA CFTICTTIGGTA CANAANAAA AATAAGGTTF TCTAATAGGT TAAGATGTTG ATTCTACAAC AAAAATACCO AGGTOCTOCT 1CTTTTTTCT0 COCATCCUTT mmmore TCCCACGTTA TCGTGGRAGT AGGICTICGCO ACARAGGAC TCCACGACGA AGANANAGAC CAACAGGAAA OTTOTOCCTTT CHECTEGOTIC DAGGAGCCAG ATACATACCC TATOTATOOG AAAAAAACAC CAATTTCAGG ACCACCTTCA TOCAGAGGGG TITITATOOC GITAMAGICC CATCATATAG CACCACCGCA GANTAAGCCA AGATTATCCA COCCCCCCT AMCCCTCCT THIGHCHANCT CTACAGCCAA AGAACAGATA ATGGCATTIG ACCINETTICA GATGICUGIT TETTGICTAT TACCGTAAAC AAAAATCTGC GAACATTAAC ATANAATGCA GMCCACCAC CFTGGTGGTG **GCC1GTCTTA** CCACCCACAG GCCCGGGGGA TTTGGGAGGA AMMICCIAIT CACTAAAAA TGACOTAACO ACTOCATION TTATTCCAAA THEAGGSTON ATCTCCTCTA TANACATTCC TITITAGACO CTTOTAATTO ACCONCCATG TATTITIACGE COCACACACAT COTOCOCTOTO ACTRCCGTTT ACATCATCAA ATTITOTANGO TTTTCCATA **GREGIGGEOF** TCTAGTAGTT CENCICIANCE GRECCOSTEC ACTICITATIA GCACCITICAG ACGIGECTOS TEGEGECGOT GAAGGGECOG COCCUMANA AGAMACACA TECTAGECAT CITICATGENO ATANAGACAG GTAAGETECG GACCGANATA CTCAPTERTY COTTITION GIGITICANCO ACTITACICAD TAGAGGAGAT CANATCCCGA ATATTAAGTC CCACCATTGT GTTTAGGGCT TATAATTCAG GCCGGTAACA TTCANAGCO GCACTOTCTG GACATATTCT ANOTITITEDS TOCACOGACC AGGGCOSCCA CTTCCCCGCC ATACTICGAM CTATOCTAAC CAGCCTACCC CCCATCTAAG CTTGTTGCAT GGGGGGAT CCCGCCGCTA CGAGTACGTC TATTICCGTC CATTCGAGGC MCATTAGAA TTGTAATCTT TTAMANGCA AATTTTTCGT CTGGCTTTAT AACACCTIGNA TTGTGGACTT ACTANAMAG TCATTETTE TCAMATIGIC AGGAAGTACG CORCGACGGG ACTATTOTAG TYPATAACATC GTTTTTTT CTANGONGAG GATCCCTCTC COCTACATTC GAACAACGTA TICTICEATAR ACACAMATA ANATARCANA ANANCATITA THATTETTT TITTETAMT AAAAAACTG GTCACCGTGA ATAGGAGAGA ANACACATA ACGRETCICE CATATATATE CCANNANCE CACANCTICC CCTCACAGAC CTGTATAAGA TAGCCAGTCA CGATTITICG TATACACTAT OFFICIALITICS CTATATATAG CCGATTTCCC AAGTCCCACT CAGTGGCACT **GCTANNAGC** CACACCTANGE Tradecoc: FTA فتنتينينين GGANGACICTG GAAGAACCAT MATCHCCANT TATCCTCTCT TITITITEAC ATCOSTCAGE CCATAACACT OGTATTGTCA TCCACAGCGA CCTTCTCGAC COCTINANCE TCCTTCATCC פאניארכטנואכ TCTCTTAGACG CAGAGACCAG TATORDECTIC GATACGATIG GICGCATCGG ACCATCAGTA TCTCTITTAL CCGCTACGC CGCACTGGCA AACACATCAG GTTGATTCAC CAACTAAGTG AACAAAATTA ACAGCGGCAG GTCGCGMAGG TGTCGCCGTC CAGTGTANA ANGORCEMO CCTACGCCCA GAMACGAMAG aconococts ocarococcs criticatific ATCCTCCATA TIGITITANT TICCCOSTIC TTARGTCCAR OCCITOACCGT IGGATGTGTA AGCAAGACGC TCAGTGTGTG CCCTCCTCCC GCCCGARGOG ARGCCARCCC OTGCAGGTTC ACCTGCATTT TATCTCTAAG ATAGAGATTC TGATTGCAAA AATTCAGGTT CGTGCAGGTC ACAGATETE CTATGTAMG GATACATTIG COCONTINUES **TCCGGTDDCG** TCCACCTAAA CAGACOCCCA AAGACGTATT TICTICTAGEC TACCAGGTAT remicensi **GCCCATIGCCG** CAOCGCTTCC GTCACATITI CHICACATA DAAGAGITTAT ACTAACCTTT CCCCACTICITY COTTCGGGTT CCAAGCCCAA ACTICACACAC CGCGCTCCCC CACCACCANG TGAACATAAT COCOTTOACA ACAGCCCCCA OPETIOCOCOF GACCGACTAC CTGCCTGATG AGACTCCOTA TCTGAGCCAT TOTOGOGGE GANCANCATA CFICTIOTAL CANTCAGTCA FACTTCTAGA TAATTCACTT PECANANGE ANCOCCET BECOGRAPTIC OPCOCITAGE **OFFINATIONS** TOGICCACGC AAGCATCCAG GCGCCCCTG ATGAAGATCT ATTAAGTGAA TTTGCCGGGA ATCTCGCCAC TAGAGCGGTG OCCOUNTIF CAGCGAATCA CAGGGCCAGC ACCARGINGE COCCOCCCC TCGTTCTGCG ATAMOCATAR DOCTOTION **PCCCGCTCCA** DOCACCAGET CCOTOOTCGA CCCAGAMAC AATAATTCTC TATTANGAG CONCECTION ATACTOTOCO DOCAMBOCT CCGTTTCGGA FATTCOTATE TCATAATOTA **AGTATTACAT** AGACAACATT CROTTOTAL CTCNACCAAA GACTICOTIT PTCOTAGOTC ACCTACACAT **EGOTTTTCCO** CAGCCTCANG TATOACAGGC **PCTCAAACAT** GAGITITOTA COCCOACC 35201 35101 34501 34901 34401 34601 35001 34101 34201 34301 34701 33601 33701 33901 34001 34801 33801

Figure 15V

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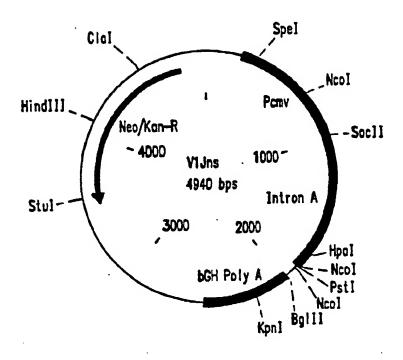
35301	CATHTITARGA	AAACTACAAT	TCCCAACACA	TACANGTTAC TYCYCCCTAA ATGITCAATG ACACCAGATT	TY COTT CCT AND AND COTT AND C	AACCTACGTC	ACCOGCCCC	TTCCCACGCC	CCGCGCCACG	TCACAMACTC ACTICTTOAG
•						Pad	el Fenfil			
35401	CACCCCCTCA	TTATCATATT	GCCTTCAATC	CANANTANGS	TATATTATTO	ATCTACAATT	TTANGAATTC	GCATCTGCGA CCTAGACGCT	COCCAGOCTO	GATGGCCTT '
35501	CCCATTATOA	AAGAAGAGCG	TTCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	ATCGCGATGC	CCGCGTTGCA	CCCCATGCTO	TCCAGGCAGG AGGTCCGTCC	TAGATTACGA	CCATCAGGGA	CACCTTCAAD
35601	COCCACCANAN	GGCCAGGAAC	CCTTAAAAAGG	CCCCOTTGCT	GCCGTTTTTC	CATAGRETIC	GCCCCCCTGA	CCACCATCAC	ANAMATECIAC TTTTTAGCTO	CCTCAAGTCA.
35701	CHECACEGER	AACCCCACAG TYGGGCTGTC	GACTATAAAG	NTACCAGGCO	TTTCCCCTG AAAGTGGGAC	CANCENCECT	CCACCCCAGA	CCTUTTCCGA	CCCTGCCGCT	TACCCEATAC ATGCCCTATE:
35801	CTOTCCOCCF	PICTCCCPTC ANGAGGIANG	OCCTICGCAC	CCCCANACAG	ATARCTCACG TATCGAGTGC	CTCTARGTAT	CTCANTTCGG	TOTAGGTCOF	TCCCTCCAAG ACCGAGGITC	CTCGCCTCT.
35901	TOCACGAACC	CCCCOTTCAG	CCCGACCGCT	CCCCCTTATC	CCCATTGATA	CCTCTTGAGT	CCAACCCGGF	AAGACACGAC TYCTGTGCTG	TTATCOCCAC AATAGCGGTG	TOOCAGCAG". ACCGTCGTC
36001	CACTGGTAAC	AGGATTAGCA TECTAATEGT	GAGCGAGGTA	TOTAGGCGGT ACATCCGCCA	CCATCACACT	TCTTCAAGTO AGAACTTCAC	GTOGCCTAAC	TACOGCTACA	CTAGANCCAC DATCTTCCTO	AGTATTTGGT TCATAAACCA
36101	ATCTOCGCTC	TOCTONA OCC ACGACTTCGG	AGTTACCTTC TCANTOGANG	CCTTTTTCTC	THE STANGETE NACEATERAS	TTGATCCGGC AACTAGGCCG	AAACAAACCA	CCCCTOOTAG	CCCTCCTTT	THIGHTING.
36201	AGCAGCAGAT TCGTCGTCTA	TACGCGCAGA	AAANANGGAT TTTTTTCCTA	CTCAAGAAGA GAGTTCTTCT	TCCTTTGATC AGGAAACTAG	TTTTCTACGG	GENCTICACIOC CCAGACTIRCO	TCAGTOGNAC AGTCACCTTO	GAAAACTCAC	GITINGGGN'I CAAITICCCTA
36301	AAACCAGTAC	AGATTATCAA	AAAGGATCTT	CACCTAGATC	CTTTTAMATC	AATCTAAAGT	ATATATGAGT TATATACTCA	AAACTTGGTC	TOACAGTTAC	CAATCCTT.W GTINCUAATI
36401	TCAGTGAGGC AGTCACTCCG	ACCTATCTCA	OCGN1CTGTC CGCTAOACAG	TATTACOME	ATCCATAGIT	OCCTONCTEC CASACTONGO	CCCTCCTCTA	GATAACTACG	ATACGGGAGO TATGCCCTCC	GCTTACCATY CCANT
36501	TOGCCCCAGT	GCTGCAATGA CGACGTTACT	TACCOCGAGA	CCCACGCTCA	CCCCCTCCAG	AFFFAFCAGC TAAAFAGTCG	AATAAACCAG TTATTTGGTC	CCACCCCCAA GOTCCCCCTT	GOCCOBACO	CAGNAGEGGE
36601	CCTCCACTT	TATCCGCTTC ATAGGCGGAG	CATCCAGACT	ATTAATTCTT	GCCGGGANGC CGCCCTTCG	THANGTHANGT	AGTTCGCCAG TCAAGCGGTC	TTAATAGTTT	COCOTTOCAA	OTTCCCATTG CANCOGTAAC
36701	CTACAGGCAT	CONCENCIA	CCCTCGTCGT CCCAGCAGTA	TTOGTATGGC AACCATACCG	TTCATTCAGC AAGTAAGTCG	ACCCCTACCC	AACGATCAAG	OCCAOTTACA COCTCAATOT	TCATCCCCCA	TOTTOTOCAA ACAACACGTT
36801	ANAMOCOGIT	AGCTCCTTCO TCGAGGAAGC	GICCICCOAT	COTTACTICAGA	AGTAAGTING TCATTCAACC	CCGCAGTGTT	ATCACTCATO	OTTATOCCAG CANTACCOTC	CACTGCATAA	TTCTCTTACT
36901	GTCATCCCAT CAGTACGGTA		CHTHCTGRO	ACTRICATEMENT TRACCACTEA	ACTUANCOAN	CACTANGACT	GAATAGTGTA	MOCCOCTOG (GAGTTIGCTCT	ACCCCGCCCT

figure 15W

PMRKAd5gag MER682

37001		TANTACCOCO	CCACATARCA	GACTITIAN	ACTUAL TREATE	ATTRACAMORC	GENCHICOCO	CCGANAACTC	TCANCGATCT	TACCTICACT
		ATTATOCCCC	GITGIECCE ATTATICICE CONCIANCIA CITINAAITT TIACCACTAS TAACCITITIC CAACAAGGC COCTITICAG ACTICCTAGA ATCCAAA	CFTCAAATTF	TEACCACTAG	TAACCITITIG	CAACAAGGCC	COCTTTTCAG	NGFICCTNGA	ATCCC CACA
37101	GAGATCCAGT	TCGATCITAAC	ONGANCEMENT ICCONTINUAC CINCITCONIC NOTCANICINA TETRINICAL CITITAGENT CACENICITA TETRICICAS CARAACAGO AMISCANANT PERTABBARA AGENERATE GENERICARIO RESERVINE AGAMENTER CANANTISMA CITARCICIA AGACCACTO GITTITICACO PICCUITATIA	ACITCAACITGA	ACAACTECTA	CANANTGAMA	CACCAGGGT	TCTCCCCTCAG	CARMACAGO	AACCCAAAAT
37201	CCCCANAAA	AGGGNATAAG	OCCOCANAA AGGINITAG OCCACACACA ANTERTIAN TACICATACT CITCCITTIT CANTALIAL GARGATITA TCAGGITTAT TCTCTCATCA COCOCITITI TCCCTTATIC CCCCACACACACACACACACACACACACACACACACA	ANATOTTICAN	TACTICATACT	CHECHTIT	CAATATTATT	GAAGCATTTA CTTCGTAAAT	TCAGESTIFAT AGTCCCAATA	TCTCTCATCA ACAGAGTACT
37301	GCGGATACAT CGCCTATGTA	ATTTOAATGT TAAACTTACA	GOGGATACAT ATTTGAATGT ATTTAGAAA ATAAACAAAT AGGGTTTCG GGGATATTC CCCGAAAAA GCACGTGAA GCATATATA: GGCCTATGTA TAAACTTACA TAAATCTTT TATTGTTTA TCCCCAAAXC GCGGGAAAA GGGCTTTTCA CGGGGACTG CAGATTCTTT GGTAATAATA	ATAMACAMAT	AGGIPSTTTCC TCCCCANGGC	CCTCTATTRC GCGTGTAAAG	CCCCANANGE	OCCACCTGAC COGTGGACTG	GTCTAAGAAA	CCATTATTA: GCTAATAATA
						Bamed	Feofil			
37401		ACCTATABAA TGGATATTTT	CATGACATTA ACCTATAGAA ATAGACGTAT CACGACGCC TTICGICTIC ANGANTICGA TUGGAGTTUT TAGT (SEQ ID NO: 27) GIACTOTAGA TGGATATTIT TATCCGCATA GTGCTCCTGG AAAGAGAGAG TTCTTAAACCT AGGCTTAAGA ATTA (SEQ ID NO: 28)	CACGACACCC GTGCTCCCTCG	TTICGICTTC AAAGCAGAAG	AAGAATTGGA TUG TTCTTAAGCT AGG	TU CCANATTET ACCEPTANCA	TANT (SEQ	ID NO: 27) ID NO: 28)	

Figure 15X



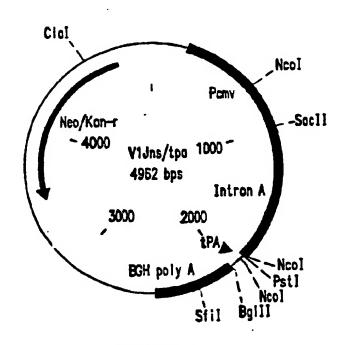


FIGURE 16

GCAGTGGCCCCTGACTGAGGAGAAGATCAAGGCCCTGCTGGAAATCTGCACTGAGATGGAGAAGGAGGGCCAAAATCTCCA sGInTrpProLeuThrGIuGIuLysIIeLysAIoLeuVoIGIuIIeCysThrGIuMelGIuLysGIuGIyLysIIeSerL 30 40 50

ACATTGGCCCCCAGAACCCCCTACAACACCCCTGTGTTTGCCATCAAGAAGAAGAACACCACCAAGTGGAGGAAGCTGGTG
yslieGiyProGiuAsnProTyrAsnThrProVoiPheAiolieLysLysLysAspSerThrLysTrpArgLysLeuVoi
60 70

GACTICAGGGAGCTGAACAAGAGGACCCAGGACTTCTGGGAGGTGCAGCTGGGCATCCCCCACCCCGCTGGCCTGAAGAA AspPheArgGluLeuAsnLysArgThrGlnAspPheTrpGluVolGlnLeuGlylleProHisProAloGlyLeuLysLy 80 90 100

GAAGAAGTCTGTGACTGTGCTGGCTGTGGGGGATGCCTACTTCTCTGTGCCCCTGGATGAGGACTTCACGAAGTACACTG slyslysSerVolThrVolLeu<u>Alo</u>VolGlyAspAloTyrPheSerVolProLeuAspGluAspPheArglysTyrThrA 110 120 130

CCTTCACCATCCCCTCCATCAACAATGAGACCCCTGGCATCAGGTACCAGTACAATGTGCTGCCCCAGGGCTGGAAGGGC loPheTnrlleProSerileAsnAsnGluThrProGlylleArgTyrGlnTyrAsnVolLeuProGinGlyTrpLysGly 140 150

TCCCCTGCCATCTCCAGTCCTCCATGACCAAGATCCTGGAGCCCTTCAGGAAGCAGAACCCTGACATTGTGATCTACCA SerProAlollePheGinSerSerMetThrLyslleLeuGluProPheArgLysGinAsnProAsplleVollleTyrGl 160 170 180

TGCTGAGGTGGGGCCTGACCACCCTGACAAGAAGCACCAGAAGGAGCCCCCCTTCCTGTGGATGGGCTATGAGCTGCAC euleuArgTrpGlyleuThrThrProAsplysLysHisGInLysGIuProProPheleuTrpMetGlyTyrGIuLeuHis 220 230

CCCGACAGTGGACTGTGCAGCCCATTGTGCTGCCTGAGAAGGACTCCTGGACTGTGAATGACATCCAGAAGCTGGTGGG ProAsplysTrpThrVoiGinProlieVoileuProGlulysAspSerTrpThrVoiAsnAsplieGinLysLeuVoiGI 240 250 260

CAAGCTGAACTGGGCCTCCCAAATCTACCCTGGCATCAAGGTGAGGCAGCTGTGCAAGCTGCTGAGGGGCACCAAGGCCC yLysleuAsnTrpAloSerGin]ieTyrProGiyIieLysVoiArgCinLeuCysLysleuLeuArgGiyThrLysAloL 270 280 290

FIGURE 17A

GGGGTGTACTATGACCCCTCCAAGGACCTGATTGCTGAGATCCAGAAGCAGGGCCAGGGCCAGTGGACCTACCAAATCTA GlyVoiTyrTyrAspProSerLysAspLeulleAloGiulleGlnLysGlnGlyGlnGlyGlnTrpThrTyrGlnlleTy 320 330 340

CCAGGAGCCCTTCAAGAACCTGAAGACTGGCAAGTATGCCAGGATGAGGGGGGCCCACACCAATGATGTGAAGCAGCTGA rGInGIuProPheLysAsnLeuLysThrGIyLysTyrAloArgMetArgGIyAIoHisThrAsnAspVoiLysGInLeuT 350 370

CTGAGGCTGTGCAGAAGATCACCACTGAGTCCATTGTGATCTGGGGCAAGACCCCCAAGTTCAAGCTGCCCATCCAGAAG hrGluAloVolGlnLyslleThrThrGluSerlleVollleTrpGlyLysThrProLysPheLysLeuProlleGlnLys 380 390

GGTGAAGCTGTGGTACCAGCTGGAGAAGGAGCCCATTGTGGGGGGCTGAGACCTTCTATGTGGCTGGGGCTGCCAACAGGG uVollysleuTrpTyrGinLeuGiuLysGiuProileVolGlyAioGiuThrPheTyrVolAioGlyAioAloAsnArgG 430 440 450

AAGACTGCCCTCCAGGCCATCTACCTGGCCCTCCAGGACTCTGGCCTGGAGGTGAACATTGTGACTGCCTCCCAGTATGC
LysThrAloLeuGInAlolleTyrLeuAloLeuGInAspSerGlyLeuGluVolAsnIleVolThrAloSerGInTyrAl
480
490
500

CCTCCCCATCATCCACCCCCACCCTGATCAGTCTGAGTCTGAGCTCGTGAACCAGATCATTGAGCAGCTGATCAAGAACG cleuGiyiieiieGinAioGinProAspGinSerGiuSerGiuLeuVoiAsnGinIielieGiuGinLeuIieLysLysG 510 520 530

AGAAGGTGTACCTGCCTGCCTGCCCACAAGGCCATTGGGGGCAATGAGCAGGTGGACAAGCTGGTGTCTGCTGGC
IULysVolTyrleuAloTrpVolProAloHislysGlyIleGlyGlyAsnGluGlnVolAsplysLeuVolSerAloGly
540
550

ATCAGGAAGGTGCTGTTCCTGGATGGCATTGACAAGGCCCAGGATGAGCATGAGAAGTACCACTCCAACTGGAGGGCTAT
1!eArgLysVoileuPheleuAspG!y1!eAspLysA!oG!nAspG!uHisG!uLysTyrHisSerAsnTrpArgA!oMe
560 570 580

FIGURE 17B

GGCCTCTGACTTCAACCTGCCCCCTGTGGTGGCTAAGGAGATTGTGGCCTCCTGTGACAAGTGCCAGCTGAAGGGGGAGG tAloSerAspPheAsnLeuProProVolVolAloLysGluIleVolAloSerCysAspLysCysGlnLeuLysGlyGluA 590 600 610

GCTGTGCATGTGGCCTCCGGCTACATTGAGGCTGAGGTGATCCCTGCTGAGACAGGCCAGGAGACTGCCTACTTCCTGCT AlovolHisVolAloSerGlyTyrIleGluAloGluVollleProAloGluThrGlyGlnGluThrAloTyrPheLeuLe 640 650 660

GAAGCTGGCTGGCAGGTGGCCTGTGAAGACCATCCACACTGCCAATGGCTCCAACTTCACTGGGGCCACAGTGAGGGCTG
uLysLeuAloGlyArgTrpProVolLysThrlleHisThrAloAsnGlySerAsnPheThrGlyAloThrVolArgAloA
670 680 690

CCTGCTGGTGGGCTGGCATCAAGCAGGAGTTTGGCATCCCCTACAACCCCCAGTCCCAGGGGGTGGTGGCCTCCATGAAC
IoCysTrpTrpAioGlylieLysGInGiuPheGlylieProTyrAsnProGinSerGinGlyVoIVoIAIoSerMelAsn
700 710

AAGGAGCTGAAGAAGATCATTGGGCAGGTGAGGGACCAGGCTGAGCACCTGAAGACAGCTGTGCAGATGGCTGTGTTCAT LysGluLeuLysLyslielleGlyGlnVolArgAspGlnAloGluHisLeuLysThrAloVolGlnMetAloVolPhell 720 730 740

CCACAACTTCAAGAGGAAGGGGGGCATCGGGGGGCTACTCCGCTGGGGAGAGGATTGTGGACATCATTGCCACAGACATCC
eHisAsnPheLysArgLysGlyGlylleGlyGlyTyrSerAloGlyGluArglleVolAsplleIleAloThrAsplleG
750
770

AGACCAAGGAGCTCCAGAAGCAGATCACCAAGATCCAGAACTTCAGGGTGTACTACAGGGACTCCAGGAACCCCCTGTGG
InThrLysGIuLeuGinLysGInlieThrLysIleGInAsnPheArgVoITyrTyrArgAspSerArgAsnProLeuTrp
780
790

AAGGCCCTGCCAAGCTGCTGTGGAAGGCGGACGGGGTGTGGTGATCCAGGACAACTCTGACATCAACGTGGTGCCCAG LysGtyProAtoLysLeuLeuTrpLysGtyGtuGtyAtoVotVotIteGtnAspAsnSerAspIteLysVotVotProAr 800 810 820

AAACCCCCCCCACATC? (SEQ ID NO: 3) Xx Ball (SEQ ID NO: 4)

FIGURE 17C

(within SEQ 10 NO: 7) (within SEQ 10 NO: 8) RoserGiul leSerAtoProlleSerProlleGiuThrVolProVolLysLeutysProGlyMetAspGly 20 10 COASIGNOAT CTOOSCOCCATOTOCCCATTCAGACTGTOCCTGTGAAGCTGAACCCTCAACCTTGCATGGC

FIGURE 18

พา	- ATG GGT GGC AAG TGG TCA AAA CGT AGT GTG CCT GGA TGG TCT	-42
OPT	- ÁTG GGC GGC ÁÁG TGG TĆC ÁÁG AĞG TCC GTG CCC GGC TGG TĆC M G G K W S K R S V P G W S	-14
WT	- ACT GTA AGG GAA AGA ATG AGA CGA GCT GAG CCA GCA GCA GAT	-84
OPT	- ÁCC GTG ÁGG GÁG ÁGG ÁTG ÁGG AGG GCC GÁG CCC GCC GCC GAC T V R E R M R R A E P A A D	-28
WT	- AGG GTG AGA CGA ACT GAG CCA GCA GCA GTA GGG GTG GGA GCA	-126
OPT	- ÁĞĞ ĞTĞ ÁĞG AĞG ÁCC ĞAĞ ČCC ĞCC ĞCC ĞTĞ ĞĞC ĞTĞ ĞĞC GCC R V R R T E P A A V G V G A	-42
WT	- GTA TCT CGA GAC CTG GAA AAA CAT GGA GCA ATC ACA AGT AGC	-168
OPT	· GTG TCC AGG GÁC CTG GÁG ÁÁG CÁC GGC GCC ÁTC ÁCC TCC TCC V S R D L E K H G A I T S S	-56
WT	- AAT ACA GCA GCT ACC AAT GCT GAT TGT GCC TGG CTA GAA GCA	-210
OPT	- ẢÁC ÁCC GẮC ÁCC ÁÁC ÁÁC ÁCC GÁC TẬC ÁCC TẬC ĆTG GÁG ÁCC N T A A T N A D C A W L E A	-7 0.
WT	- CAA GAG GAT GAG GAA GTG GGT TTT CCA GTC AGA CCT CAG GTA	-252
OPT	- CÁG GÁG GÁC GÁG GÁG GTG GGC TTC CĆC GTG ÁGG CĆC CAG GTG Q E D E E V G F P V R P Q V	-84
WT	- CCT TTA AGA CCA ATG ACT TAC AAG GGA GCT GTA GAT CTT AGC	-294
OPT	- CCC CTG ÁGG CCC ÁTG ÁCC TÁC ÁÁG GGC GCC GTG GÁC CTG TCC PLR PM T Y K G A V D L S	-98
WT	- CAC TTT TTA AAA GAA AAG GGG GGA CTG GAA GGG CTA ATT CAC	-336
OPT	- CÁC TÍC CTG ÁÁS GÁG ÁÁG GÉC GÉC CTG ÁG CÁC H F L K E K G G L E G L I H	-112
WT	- TCA CAG AAA AGA CAA GAT ATC CTT GAT CTG TGG GTC TAC CAC	-378
OPT	- TCC CAG AAG AGG CAG GAC ATC CTG GAC CTG TGG GTG TAC CAC S Q K R Q D I L D L W V Y H	- -126
WT	- ACA CAA GGC TAC TTC CCT GAT TGG CAG AAC TAC ACA CCA GGG	-420
OPT	- ACC CAG GGC TAC TTC CCC GAC TGG CAG AAC TAC ACC CCC GGC T Q G Y F P D W Q N Y T P G	-140

FIGURE 19A

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WT	- CCA GGA ATC AGA TTT CCA TTG ACC TTT GGA TGG TGC TTC AAG -462	
OPT	- CÉC GẬC ÁTC ÁĞG TTC CÉC CTĞ ÁCC TTC ĞĞC TĞĞ TGC TTC AAĞ PGIRFPLT FGWCFK -154	
WT	- CTA GTA CCA GTT GAG CCA GAA AAG GTA GAA GAG GCC AAT GAA -504	
OPT	- CTG GTG CCC GTG GAG CCC GAG AAG GTG GAG GAG GCC AAC GAG L V P V E P E K V E E A N E -168	
WT	- GGA GAG AAC AAC TGC TTG TTA CAC CCT ATG AGC CAG CAT GGG .546	
OPT	- GGC GAG AAC AAC TGC CTG CTG CAC CCC ATG TCC CAG CAC GGC G E N N C L L H P M S Q H G -182)
WT	- ATA GAG GAC CCG GAG AAG GAA GTG TTA GAG TGG AGG TTT GAC -588	}
OPT	- ATC GAG GAC CCC GAG AAG GAG GTG CTG GAG TGG AGG TTC GAC 1 E D P E K E V L E W R F D -196	5
WT .	- AGC AAG CTA GCA TTT CAT CAC GTG GCC CGA GAG CTG CAT CCG -63)
OPT	- TCC AAG CTG GCC TTC CAC CAC GTG GCC AGG GAG CTG CAC CCC S K L A F H H V A R E L H P -21	0
WT	- GAG TAC TAC AAG GAC TGC TGA (SEQ ID ND:30) -65	1
OPT	- GAG TAC TAC AAG GAC TGC TAA (contained within SEQID NO:9) E Y Y K D C (SEQID NO:10) -21	6

FIGURE 19B

VIJns/nef

Srf1 Bg111
. . . . CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGCAGAICIGCCTTCTAGTTGCCAGC (SEQ 10 ND: 38)
H P E Y Y K D C * (Contained within SEQ 10 ND: 10:

V1Jns/nef(G2A.LLAA)

Psti Catgrictiticigicacicottraggaticigicacic atg gcc ggc ang tgg tcc ang agg tcc gtg ccc . M A G K W S K R S V P

SrfI BOILI CAC CCC GAG TAC TAC AGG GAC TGC TAA AGCCCGGGCAGAICIGCCTTCTAGTTGCCAGC (SEQ 10 NO: 39) H P E Y Y K D C * (contained within SEQ ID NO:14)

VlJns/tpanef & VlJns/tpanef(LLAA)

CATGGGTCTTTCTGGAGTCACCGTCCTTATATCTAGATCACC ATG GAT GCA ATG ANG AGA GGG CTC TGC TGT GTG NO A N K R G L C C V

CTG CTG CTG TGT GGA GCA GTC TTC GTT TCG CCC AGC GAG Δ IC TCC AAG AGG TCC GTG CCC ...

SrfI BgJII

CAC CCC GAG TAC TAC AAG GAC TGC TAA AGCCCGGGGAGICIGCTGTGCCTTCTAGTTGCCAGC (SEQ ID NO: 40)

H P . E Y Y K D C * (contained withon SEQ ID NO: 16)

FIGURE 20

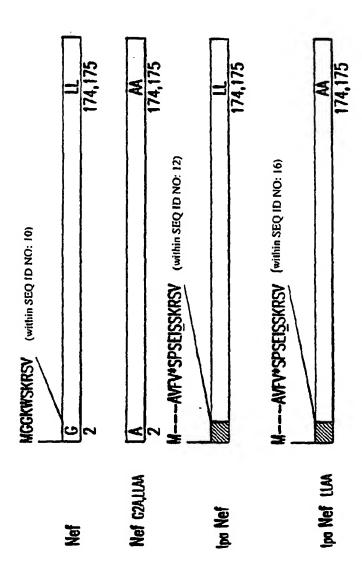


FIGURE 21

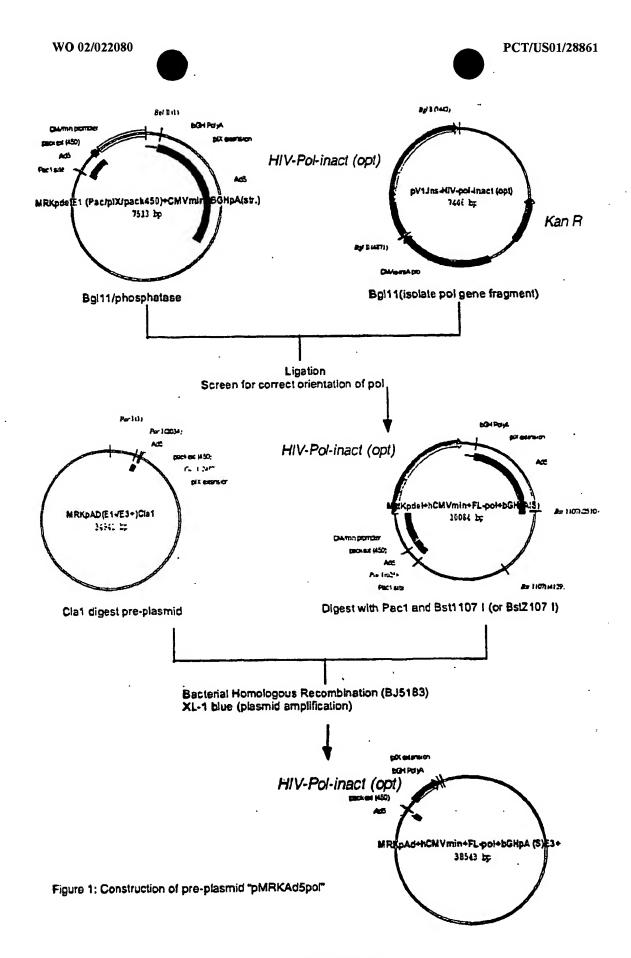
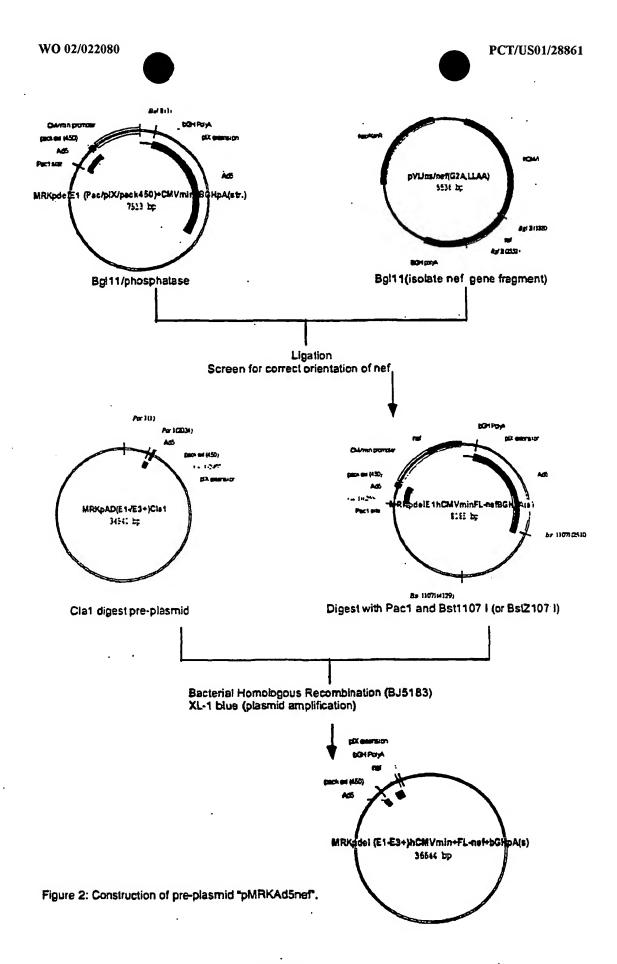


FIGURE 22



Comparison of Clade B vs. Clade C Anti-gag T Cell Responses in Clade B HIV-Infected Subjects

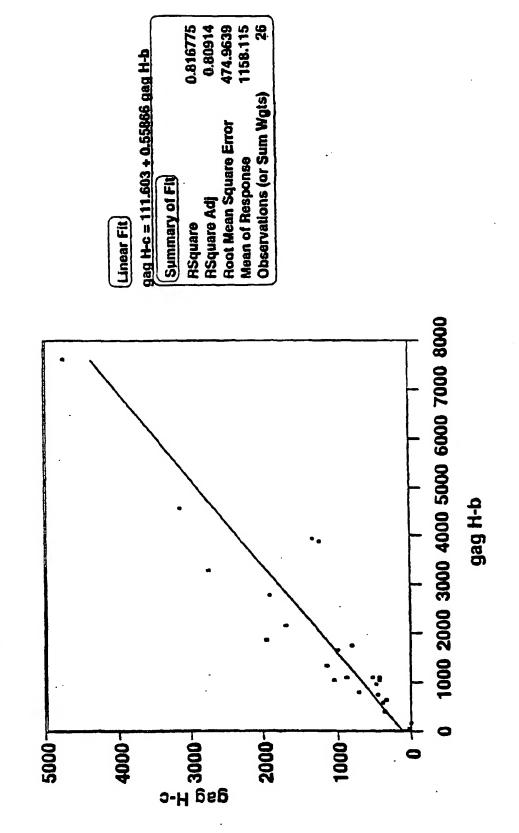
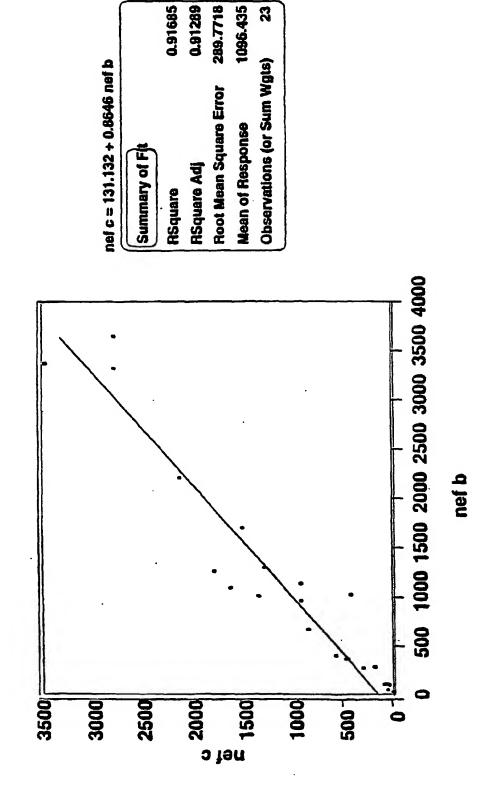


FIGURE 25

23

Comparison of Clade B vs. Clade C Anti-nef T Cell Responses in Clade B HIV-Infected Subjects





1	CATCATCAAT	AATATACCTT	ATTTTGGATT	GAAGCCAATA	TGATAATGAG
•	GTAGTAGTTA				
51	GGGGTGGAGT				
	CCCCACCTCA	AACACTGCAC	CGCGCCCCCGC	ACCCTTGCCC	CGCCCACTGC
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
	ATCATCACAC				
151	GCGACGGATG				
	CGCTGCCTAC	ACCGTTTTCA	CIGCAAAAAC	CACACGCGGC	CACATGIGIC
201	GAAGTGACAA	TTTTCGCGCG	GTTTTAGGCG	GATGTTGTAG	TAAATTTGGG
	CTTCACTGTT	AAAAGCGCGC	CAAAATCCGC	CTACAACATC	DODKAKTTEA
251				GGGAAAACTG CCCTTTTGAC	
	GCATIGGCIC	ATTCTAAACC	GGIAAAAGCG	CCCITITUAC	TIMITOTOCI
301	AGTGAAATCT	GAATAATTTT	GTGTTACTCA	TAGCGCGTAA	TATTTGTCTA
	TCACTTTAGA	CTTATTAAAA	CACAATGAGT	ATCGCGCATT	ATAAACAGAT
					> 00m0mmmm
351					AGGTGTTTTT TCCACAAAAA
	CCCGGCGCCC	CIGAAACIGG	CAAAIGCACC	TCTGAGCGGG	1ccnc/down
401	CTCAGGTGTT	TTCCGCGTTC	CGGGTCAAAG	TTGGCGTTTT	ATTATTATAG
	GAGTCCACAA	AAGGCGCAAG	GCCCAGTTTC	AACCGCAAAA	TAATAATATC
			>	C2020C2022	mamcmacam
451					TATGTACATT ATACATGTAA
•	(60066001	AGGIAACGIA	IGCARCAIAG	diainoini.	n mont of the
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	TGTTGACATT	GATTATTGAC
	ATATAACCGA	GTACAGGTTG	TAATGGCGGT	ACAACTGTAA	CTAATAACTG
	ma	m,	mm x c c c c c c c c	· አጥጥአር መጥር አጥ	AGCCCATATA
551					TCGGGTATAT

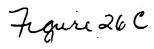
601					TGGCTGACCG
	ACCTCAAGGC	GCAATGTATT	GAATGCCATI	TACCGGGCGG	ACCGACTGGC
651	000000000	CCCCCCC X TOTAL	CACCOCAADA		TTCCCATAGT
621					AAGGGTATCA
					TATTTACGGT
	. TTGCGGTTAT	CCCTGAAAGG	TAACTGCAG	r TACCCACCTC	: ATAAATGCCA
751	3	CTTCCCACTA	CATCAACTC	P	AAGTACGCCC
751					TTCATGCGGG
			•		•
801					ATGCCCAGTA
	GGATAACTGC	: AGTTACTGCC	ATTTACCGG	g CGGACCGTAJ	1 TACGGGTCAT
051	CANCACCIONI	י יייבוריני אַ ריינייייי	CTACTTCC	ል ርሞልሮልጥርሞል(GTATTAGTCA
671					CATAATCAGT

7 i jure 26A

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901	TCGCT	CATGGTGATG	CGGTTTTGGC	AGTACATCAA	GCGTGGA	
	AGCGATAATG	GTACCACTAC	GCCAAAACCG	TCATGTAGTT	ACCCGCACCT	
951					TTGACGTCAA	
					AACTGCAGTT	
1001					AAATGTCGTA .	
					TTTACAGCAT	
1051					ACGGTGGGAG	
		GGGTAACTGC				
1101	GTCTATATAA					
4454		CGTCTCGAGC				
1151	CCATCCACGC					
7201		ACAAAACTGG				
1201	TCCGCGGCCG				TGCCAAGAGT ACGGTTCTCA	
	AGGCGCCGGC	CCITGCCACG	TAACCTTGCG	CCIMAGGGGC	ACGGITCICA	
1251	GAGATCTACC	ATGGCCCCCA	TCTCCCCCAT	TGAGACTGTG	CCTGTGAAGC	
	CTCTAGATGG	TACCGGGGGT	AGAGGGGGTA	ACTCTGACAC	GGACACTTCG	
1301	TGAAGCCTGG					
		GTACCTACCG	GGGTTCCACT	TCGTCACCGG	GGACTGACTC ·	
1351		AGGCCCTGGT				
		TCCGGGACCA				
1401					CCTGTGTTTG	
1 4 5 7		TTCTAACCGG				
1451	CCATCAAGAA	CTTCCTGAGG				
1501	GAGCTGAACA					
1301		TCTCCTGGGT				
1551					CTGGCTGTGG	
1001		CCGGACTTCT				
		000000000000000000000000000000000000000			0.1000.101.00	
1601	GGGATGCCTA	${\tt CTTCTCTGTG}$	CCCCTGGATG	AGGACTTCAG	GAAGTACACT	
	CCCTACGGAT	GAAGAGACAC	GGGGACCTAC	TCCTGAAGTC	CTTCATGTGA	
1651	GCCTTCACCA					
	CGGAAGTGGT	AGGGGAGGTA	GTTGTTACTC	TGGGGACCGT	AGTCCATGGT	
1701	GTACAATGTG					
					TAGAAGGTCA	
	CCTCCATGAC					
,					GGGACTGTAA	
1801	GTGATCTACC					
	CACTAGATGG	TCATGTACCG	ACGGGACATA	CACCCGAGAC	TGGACCTCTA	

Figure 24 B

1901	CCCCCTCAC	CACCCCTGAC	AAGAAGCACC	AGAAGGAGCC	CCCCTTCCTG
1501			TTCTTCGTGG		
1951	тесатесест	ATGAGCTGCA	CCCCGACAAG	TGGACTGTGC	AGCCCATTGT
1751			GGGGCTGTTC		
2001	GCTGCCTGAG	AAGGACTCCT	GGACTGTGAA	TGACATCCAG	AAGCTGGTGG
	CGACGGACTC	TTCCTGAGGA	CCTGACACTT	ACTGTAGGTC	TTCGACCACC
2051	GCAAGCTGAA	CTGGGCCTCC	CAAATCTACC	CTGGCATCAA	GGTGAGGCAG
			GTTTAGATGG		
2101	CTGTGCAAGC	TGCTGAGGGG	CACCAAGGCC	CTGACTGAGG	TGATCCCCCT
	GACACGTTCG	ACGACTCCCC	GTGGTTCCGG	GACTGACTCC	ACTAGGGGGA
2151	GACTGAGGAG	GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGG
2131			TCGACCGACT		
	CIGACICCIC	conc rednee	TOORCOMET		111001101
2201	AGCCTGTGCA	TGGGGTGTAC	TATGACCCCT	CCAAGGACCT	GATTGCTGAG
	TCGGACACGT	ACCCCACATG	ATACTGGGGA	GGTTCCTGGA	CTAACGACTC
				m> 00> > > mom	
2251			CCAGTGGACC		
	TAGGTCTTCG	TCCCGGTCCC	GGTCACCTGG	ATGGTTTAGA	TGGTCCTCGG
2301	CTTCAAGAAC	CTGAAGACTG	GCAAGTATGC	CAGGATGAGG	GGGGCCCACA
	•••	•	CGTTCATACG		
2351			ACTGAGGCTG		
	GGTTACTACA	CTTCGTCGAC	TGACTCCGAC	ACGTCTTCTA	GTGGTGACTC
2401	тссаттетса	TCTGGGGCAA	GACCCCCAAG	TTCAAGCTGC	CCATCCAGAA
			CTGGGGGTTC		
2451			GGACTGAGTA		
	CCTCTGGACC	CTCTGGACCA	CCTGACTCAT	GACCGTCCGG	TGGACCTAGG
				macmax > aam	CMCCM2 CC2C
2501			ACCCCCCCC		
	GACTCACCCT	CAAACACTTG	TGGGGGGGG	ACCACTICGA	CACCAIGGIC
2551	CTGGAGAAGG	AGCCCATTGT	GGGGGCTGAG	ACCTTCTATG	TGGCTGGGGC
2332					ACCGACCCCG
				•	
2601					ACCAACAGGG
	ACGGTTGTCC	CTCTGGTTCG	ACCCGTTCCG	ACCGATACAC	TGGTTGTCCC
				•	
2651					GAAGACTGCC
	CGTCCGTCTT	CCACCACTGG	GACTGACTGT	GGTGGTTGGT	CTTCTGACGG
2201	0000000000	m/m x // / / / / / /		WORKED ONCO	AGGTGAACAT
2/01					TCCACTTGTA
	GAGGTEEGGT	MOW I GOVECO	GONGOICCIG	AGACCOGACC	100n01101A
2751	TGTGACTGCC	TCCCAGTATG	CCCTGGGCAT	CATCCAGGCC	CAGCCTGATC
					CTCCCACTAC



ACACTGACGG AGGGTCATAC GGGACCCGTA GTAGGTCCGG GTCGGACTAG

2851				CACAAGGGCA GTGTTCCCGT	
2901	• • • • • • • • •			CATCAGGAAG GTAGTCCTTC	
2951				ATGAGAAGTA TACTCTTCAT	
3001				CCCCCTGTGG GGGGGACACC	
3051	GATTGTGGCC	TCCTGTGACA	AGTGCCAGCT	GAAGGGGGAG CTTCCCCCTC	GCCATGCATG
3101	GGCAGGTGGA	CTGCTCCCCT	GGCATCTGGC	AGCTGGCCTG	CACCCACCTG
3151	GAGGGCAAGG	TGATCCTGGT	GGCTGTGCAT	TCGACCGGAC GTGGCCTCCG	GCTACATTGA
3201				CACCGGAGGC GGAGACTGCC	
	CCGACTCCAC	TAGGGACGAC	TCTGTCCGGT	CCTCTGACGG CCATCCACAC	ATGAAGGACG
3251	ACTTCGACCG	ACCGTCCACC	GGACACTTCT	GGTAGGTGTG	ACGGTTACCG
3301				GCCTGCTGGT CGGACGACCA	
3351				CCAGTCCCAG GGTCAGGGTC	
3401	CCTCCATGAA GGAGGTACTT			TTGGGCAGGT AACCCGTCCA	•
3451				GCTGTGTTCA CGACACAAGT	
3501	CAAGAGGAAG GTTCTCCTTC			CGCTGGGGAG GCGACCCCTC	
3551	ACATCATTGC TGTAGTAACG			AGCTCCAGAA TCGAGGTCTT	
3601	AAGATCCAGA TTCTAGGTCT			GACTCCAGGA CTGAGGTCCT	
3651	GAAGGGCCCT CTTCCGGGA			GGAGGGGGCT CCTCCCCGA	
3701	AGGACAACTC	TGACATCAAG	GTGGTGCCCA		CAAGATCATC

7 gure 26 D

3801	GGATGAGGAC	TAAAGCCCGG	GCAGATCTGC	TGTGCCTTCT	AGTTGCCAGC
	CCTACTCCTG	ATTTCGGGCC	CGTCTAGACG	ACACGGAAGA	TCAACGGTCG
3851			CCCGTGCCTT		
	GTAGACAACA	AACGGGGAGG	GGGCACGGAA	GGAACTGGGA	CCTTCCACGG
3901	ACTCCCACTG	TCCTTTCCTA	ATAAAATGAG	GAAATTGCAT	CGCATTGTCT
			TATTTTACTC		
3951	GAGTAGGTGT	CATTCTATTC	TGGGGGGTGG	GGTGGGGCAG	GACAGCAAGG
			ACCCCCACC		
4001			AGCAGGCATG		
			TCGTCCGTAC		
4051			ACTGAAATGT		
			TGACTTTACA		
4101			GGGTCTTATG		
			CCCAGAATAC		
4151			GCACCAACTC		
			CGTGGTTGAG		
4201			ATGCCCCCAT		
			TACGGGGGTA		
4251			TGGTCGCCCC		
			ACCAGCGGGG		
4301			TGTCTGGAAC		
			ACAGACCTTG		
4351			GCAGCCACCG		
			CGTCGGTGGC		
4401			TGCAAACAGT		
	_		ACGTTTGTCA		
4451					TCTTTGACCC
					AGAAACTGGG
4501					CCAGCAGGTT GGTCGTCCAA
4551	TCTGCCCTGA	AGGCTTCCTC	CCCTCCCAAT	GCGGTTTAAA	ACATAAATAA
					TGTATTTATT
4601					TGCTGTCTTT
					ACGACAGAAA
4651					GTCTCGGTCG
	TAAATCCCCA	AAACGCGCGC	GCCATCCGGG	CCCTGGTCGC	CAGAGCCAGC

Figure 26E

WO 02/022080 PCT/US01/28861

4751	GTTCAGATAC	ATGGGCATAA	GCCCGTCTCT	GGGGTGGAGG	TAGCACCACT
	CAAGTCTATG.	TACCCGTATT	CGGGCAGAGA	CCCCACCTCC	ATCGTGGTGA
4801				AGATGATCCA	
	CGTCTCGAAG	TACGACGCCC	CACCACAACA	TCTACTAGGT	CAGCATCGTC
4851	CACCCCTCCC	ССТССТСССТ	AAAAATGTCT	TTCAGTAGCA	AGCTGATTGC
4031				AAGTCATCGT	
4901	CAGGGGCAGG	CCCTTGGTGT	AAGTGTTTAC	AAAGCGGTTA	AGCTGGGATG
1,,,,				TTTCGCCAAT	
4951	GGTGCATACG	TGGGGATATG	AGATGCATCT	TGGACTGTAT	TTTTAGGTTG
4551				ACCTGACATA	
5001	CCTATGTTCC	CAGCCATATC	CCTCCGGGGA	TTCATGTTGT	GCAGAACCAC
3001				AAGTACAACA	
5051	CAGCACAGTG	TATCCGGTGC	ACTTGGGAAA	TTTGTCATGT	AGCTTAGAAG
3031				AAACAGTACA	
	0.00.0.0.0.0				
5101	GAAATGCGTG	GAAGAACTTG	GAGACGCCCT	TGTGACCTCC	AAGATTTTCC
	CTTTACGCAC	CTTCTTGAAC	CTCTGCGGGA	ACACTGGAGG	TTCTAAAAGG
5151	ATGCATTCGT	CCATAATGAT	GGCAATGGGC	CCACGGGCGG	CGGCCTGGGC
	TACGTAAGCA	GGTATTACTA	CCGTTACCCG	GGTGCCCGCC	GCCGGACCCG
5201	GAAGATATTT	CTGGGATCAC	TAACGTCATA	GTTGTGTTCC	AGGATGAGAT
	СТТСТАТААА	GACCCTAGTG	ATTGCAGTAT	CAACACAAGG	TCCTACTCTA
5251	CGTCATAGGC	CATTTTTACA	AAGCGCGGGC	GGAGGGTGCC	AGACTGCGGT
	GCAGTATCCG	GTAAAAATGT	TTCGCGCCCG	CCTCCCACGG	TCTGACGCCA
5301	ATAATGGTTC	CATCCGGCCC	AGGGGCGTAG	TTACCCTCAC	AGATTTGCAT
	TATTACCAAG	GTAGGCCGGG	TCCCCGCATC	AATGGGAGTG	TCTAAACGTA
5351					TGCGGGGCGA
					ACGCCCCGCT
5401					AGAAAGCAGG
					TCTTTCGTCC
5451					AAATCACACC
					TTTAGTGTGG
5501					CCGTCATCCC
					GGCAGTAGGG
5551					CATGTTTTCC
	ACTCGTCCCC	CCGGTGAAGC	AATTCGTACA	GGGACTGAGC	GTACAAAAGG
5601	CTGACCAAAT	CCGCCAGAAG	GCGCTCGCCG	CCCAGCGATA	GCAGTTCTTG
					CGTCAAGAAC

Figure 26 F

5701	TTTTGAGCGT	TTGACCAAGC	AGTTCCAGGC	GGTCCCACAG	CTCGGTCACC
	AAAACTCGCA	AACTGGTTCG	TCAAGGTCCG	CCAGGGTGTC	GAGCCAGTGG
5751	ጥርርጥርጥልርርር	CATCTCGATC	CAGCATATCT	CCTCGTTTCG	CGGGTTGGGG
7/1		GTAGAGCTAG			
	ACGAGATGCC	GIAGAGCIAG	GICGIAIAGA	GGAGCAMAGC	GCCCAACCCC
			5 1.0555555	maamaa, a, a	000001000m
5801		TGTACGGCAG			
	GCCGAAAGCG	ACATGCCGTC	ATCAGCCACG	AGCAGGTCTG	CCCGGTCCCA
5851		CACGGGCGCA			
	GTACAGAAAG	GTGCCCGCGT	CCCAGGAGCA	GTCGCATCAG	ACCCAGTGCC
5901	TGAAGGGGTG	CGCTCCGGGC	TGCGCGCTGG	CCAGGGTGCG	CTTGAGGCTG
•••		GCGAGGCCCG			
5951	CTCTCCTCC	TGCTGAAGCG	СТСССССТСТ	TCGCCCTGCG	CGTCGGCCAG
3331		ACGACTTCGC			
	CAGGACGACC	ACGACTICGC	guragerion	200000000000000000000000000000000000000	000000010
6001	OMA COA MINING	ACCATGGTGT	CATACTCCAC	CCCCTCCCC	CCCTCCCCT
6001		TGGTACCACA			
	CATCGTAAAC	TGGTACCACA	GTATCAGGTC	GGGGAGGCGC	CGCACCGGGA
6051		CTTGCCCTTG			
	ACCGCGCGTC	GAACGGGAAC	CTCCTCCGCG	GCGTGCTCCC	CGTCACGTCT
6101		CGTAGAGCTT			
	GAAAACTCCC	GCATCTCGAA	CCCGCGCTCT	TTATGGCTAA	GGCCCCTCAT
					•
6151	GGCATCCGCG	CCGCAGGCCC	CGCAGACGGT	CTCGCATTCC	ACGAGCCAGG
	CCGTAGGCGC	GGCGTCCGGG	GCGTCTGCCA	GAGCGTAAGG	TGCTCGGTCC
6201	TGAGCTCTGG	CCGTTCGGGG	TCAAAAACCA	GGTTTCCCCC	ATGCTTTTTG
		GGCAAGCCCC			
6251	አጥር ርርጥጥጥርጥ	TACCTCTGGT	TTCCATGAGC	CGGTGTCCAC	GCTCGGTGAC
0231		ATGGAGACCA			
	IACGCAAAGA	AIGGAGACCA	MIGGINETEG	000110110010	
6201	011110C0E0	TCCGTGTCCC	CCMNMNCNCN	CMMCACACCC	CTCTCCTCGA
6301		AGGCACAGGG			
•	CTTTTCCGAC	AGGCALAGGG	GCATATGTCT	GAACICICCG	GACAGGAGCI
					0000000000
6351	GCGGTGTTCC	GCGGTCCTCC	TCGTATAGAA	ACTCGGACCA	CTCTGAGACA
	CGCCACAAGG	CGCCAGGAGG	AGCATATCTT	TGAGCCTGGT	GAGACTCTGT
6401	AAGGCTCGCG	TCCAGGCCAG	CACGAAGGAG	GCTAAGTGGG	AGGGGTAGCG
	TTCCGAGCGC	AGGTCCGGTC	GTGCTTCCTC	CGATTCACCC	TCCCCATCGC
6451		ACTAGGGGGT			
		TGATCCCCCA			
	5				
6501	CGCCCTCTTC	GGCATCAAGG	AAGGTGATTG	GTTTGTAGGT	GTAGGCCACG
0001	CCCCCTCTTC	CCGTAGTTCC	TTCCACTAAC	CAAACATCCA	CATCCGGTGC
	JCGGGRGRAG				
	TO A COOCOCO	#####################################	CCCCCTATA	A A CCCCCTTCC	GGGCGCGTTC
0227		AAGGACTTCC			
	ACTGGCCCAC	AAGGACTTCC	CCCCOWINII	TACCCCCACC	CCCGCGCAAG

Figure 266

6651 AGTALICCCT CTGAAAAGCG GGCATGACTT CTGCGCTAAG ATTGTCAGTT TCATGAGGGA GACTTTTCGC CCGTACTGAA GACGCGATTC TAACAGTCAA 6701 TCCAAAACG AGGAGGATTT GATATTCACC TGGCCCGCGG TGATGCCTTT AGGTTTTTGC TCCTCCTAAA CTATAAGTGG ACCGGGCGCC ACTACGGAAA 6751 GAGGGTGGCC GCATCCATCT GGTCAGAAAA GACAATCTTT TTGTTGTCAA CTCCCACCGG CGTAGGTAGA CCAGTCTTTT CTGTTAGAAA AACAACAGTT 6801 GCTTGGTGGC AAACGACCCG TAGAGGGCGT TGGACAGCAA CTTGGCGATG CGAACCACCG TTTGCTGGGC ATCTCCCGCA ACCTGTCGTT GAACCGCTAC 6851 GAGCGCAGGG TTTGGTTTTT GTCGCGATCG GCGCGCTCCT TGGCCGCGAT CTCGCGTCCC AAACCAAAAA CAGCGCTAGC CGCGCGAGGA ACCGGCGCTA 6901 GTTTAGCTGC ACGTATTCGC GCGCAACGCA CCGCCATTCG GGAAAGACGG CAAATCGACG TGCATAAGCG CGCGTTGCGT GGCGGTAAGC CCTTTCTGCC 6951 TGGTGCGCTC GTCGGGCACC AGGTGCACGC GCCAACCGCG GTTGTGCAGG ACCACGCGAG CAGCCCGTGG TCCACGTGCG CGGTTGGCGC CAACACGTCC 7001 GTGACAAGGT CAACGCTGGT GGCTACCTCT CCGCGTAGGC GCTCGTTGGT CACTGTTCCA GTTGCGACCA CCGATGGAGA GGCGCATCCG CGAGCAACCA 7051 CCAGCAGAGG CGGCCGCCCT TGCGCGAGCA GAATGGCGGT AGGGGGTCTA GGTCGTCTCC GCCGGCGGA ACGCGCTCGT CTTACCGCCA TCCCCCAGAT 7101 GCTGCGTCTC GTCCGGGGGG TCTGCGTCCA CGGTAAAGAC CCCGGGCAGC CGACGCAGAG CAGGCCCCC AGACGCAGGT GCCATTTCTG GGGCCCGTCG 7151 AGGCGCGCGT CGAAGTAGTC TATCTTGCAT CCTTGCAAGT CTAGCGCCTG TCCGCGCGCA GCTTCATCAG ATAGAACGTA GGAACGTTCA GATCGCGGAC 7201 CTGCCATGCG CGGGCGCCAA GCGCGCGCTC GTATGGGTTG AGTGGGGGAC GACGGTACGC GCCCGCCGTT CGCGCGCGAG CATACCCAAC TCACCCCCTG 7251 CCCATGGCAT GGGGTGGGTG AGCGCGGAGG CGTACATGCC GCAAATGTCG GGGTACCGTA CCCCACCCAC TCGCGCCTCC GCATGTACGG CGTTTACAGC 7301 TAAACGTAGA GGGGCTCTCT GAGTATTCCA AGATATGTAG GGTAGCATCT ATTTGCATCT CCCCGAGAGA CTCATAAGGT TCTATACATC CCATCGTAGA 7351 TCCACCGCGG ATGCTGGCGC GCACGTAATC GTATAGTTCG TGCGAGGGAG AGGTGGCGCC TACGACCGCG CGTGCATTAG CATATCAAGC ACGCTCCCTC 7401 CGAGGAGGTC GGGACCGAGG TTGCTACGGG CGGGCTGCTC TGCTCGGAAG GCTCCTCCAG CCCTGGCTCC AACGATGCCC GCCCGACGAG ACGAGCCTTC 7451 ACTATCTGCC TGAAGATGGC ATGTGAGTTG GATGATATGG TTGGACGCTG TGATAGACGG ACTTCTACCG TACACTCAAC CTACTATACC AACCTGCGAC 7501 GAAGACGTTG AAGCTGGCGT CTGTGAGACC TACCGCGTCA CGCACGAAGG CTTCTGCAAC TTCGACCGCA GACACTCTGG ATGGCGCAGT GCGTGCTTCC

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Figure 26 H

7601	TCTAGGGCGC	AGTAGTCCAG	GGTTTCCTTG	ATGATGTCAT	ACTTATCCTG
7001		TCATCAGGTC			
	MGMICCCGCG	ICAICAGGIC	CCAAAGGAAC	INCINCAGIA	IGAMINGGAC
7651	TCCCTTTTTT	TTCCACAGCT	CGCGGTTGAG	GACAAACTCT	TCGCGGTCTT
	AGGGAAAAA	AAGGTGTCGA	GCGCCAACTC	CTGTTTGAGA	AGCGCCAGAA
2201	maaa ama ama	TTGGATCGGA	3.3.000000000	CCTCCCAACC	CMNNCNCCCM
7701					
	AGGTCATGAG	AACCTAGCCT	TTGGGCAGCC	GGAGGCTTGC	CATTCTCGGA
7751	AGCATGTAGA	ACTGGTTGAC	GGCCTGGTAG	GCGCAGCATC	CCTTTTCTAC
		TGACCAACTG			
	ICGINCALCI	. Griccia ic i C	CCOORCORIC		00.222.00
7801		TATGCCTGCG			
	CCCATCGCGC	ATACGGACGC	GCCGGAAGGC	CTCGCTCCAC	ACCCACTCGC
7851	CAAAGGTGTC	CCTGACCATG	ACTTTGAGGT	ACTGGTATTT	GAAGTCAGTG
,031		GGACTGGTAC			
	GITTCCACAG	GGACTGGTAC	1GAAAC1CCA	IGACCATAAA	CITCAGICAC
7901	TCGTCGCATC	CGCCCTGCTC	CCAGAGCAAA	AAGTCCGTGC	GCTTTTTGGA
	AGCAGCGTAG	GCGGGACGAG	GGTCTCGTTT	TTCAGGCACG	CGAAAAACCT
7951	እርርርርርስምም	GGCAGGGCGA	ACCTCA CATC	CTTCNACACT	እጥርጥጥጥርርር ር
7951					
	TGCGCCTAAA	CCGTCCCGCT	TCCACTGTAG	CAACTTCTCA	TAGAAAGGGC
8001	CGCGAGGCAT	AAAGTTGCGT	GTGATGCGGA	AGGGTCCCGG	CACCTCGGAA
	GCGCTCCGTA	TTTCAACGCA	CACTACGCCT	TCCCAGGGCC	GTGGAGCCTT
	00001000111				
			0000100100	> mamaama> >	> 00000mm0> m
8051		TTACCTGGGC			
	GCCAACAATT	AATGGACCCG	CCGCTCGTGC	TAGAGCAGTT	TCGGCAACTA
8101	GTTGTGGCCC	ACAATGTAAA	GTTCCAAGAA	GCGCGGGATG	CCCTTGATGG
0202 /		TGTTACATTT			
	CAACACCGGG	IGIIACAIII	CAAGGIICII	COCOCCCIAC	000,11,01,100
		•			
8151		TTTAAGTTCC			
	TTCCGTTAAA	AAATTCAAGG	AGCATCCACT	CGAGAAGTCC	CCTCGACTCG
8201	СССТССТСТС	AAAGGCCCA	GTCTGCAAGA	TGAGGGTTGG	AAGCGACGAA
0201		TTTCCCGGGT			
	GGCACGAGAC	1111000661	CAGACGITCI	ACICCAACC	1100010011
8251	TGAGCTCCAC				
	ACTCGAGGTG	TCCAGTGCCC	GGTAATCGTA	AACGTCCACC	AGCGCTTTCC
02/1	TCCTAAACTG	CCCACCMAMC	CCC 3 december	CACCCCACA	CCACTACAAC
9201					
	AGGATTTGAC	CGCTGGATAC	CGGTAAAAA	GACCCCACTA	CGTCATCTTC
8351	GTAAGCGGGT	CTTGTTCCCA	GCGGTCCCAT	CCAAGGTTCG	CGGCTAGGTC
		GAACAAGGGT			
0400		000100100		0000330000	NMCNON.001
8401					ATGACCAGCA
	AGCGCGCCGT	CAGTGATCTC	CGAGTAGAGG	CGGCTTGAAG	TACTGGTCGT
8451	TGAAGGGCAC	GAGCTGCTTC	CCAAAGGCCC	CCATCCAAGT	ATAGGTCTCT
					TATCCAGAGA
	VC11CCCA10	CICOMO			

Figure 26I

8551	GAAGAACTGG	ATCTCCCGCC	ACCAATTGGA	GGAGTGGCTA	TTGATGTGGT
				CCTCACCGAT	
	C11C11G				
8601	CAAACTACAA	CTCCCTCCCA	CCCCCCCAAC	ACTCGTGCTG	CCTTTTCTAA
9901				TGAGCACGAC	
	CTTCATCTT	CAGGGACGCI	GCCCGGCIIG	IGNOCACOAC	COMMACNII
					0000000000
8651				GGCTGTACAT	
	TTTGCACGCG	TCATGACCGT	CGCCACGTGC	CCGACATGTA	GGACGTGCTC
					•
8701				GAGTGGGAAT	
	CAACTGGACT	GCTGGCGCGT	GTTCCTTCGT	CTCACCCTTA	AACTCGGGGA
8751	CGCCTGGCGG	GTTTGGCTGG	TGGTCTTCTA	CTTCGGCTGC	TTGTCCTTGA
				GAAGCCGACG	
	0000.10000				
8801	CCCTCTCCCCT	CCTCCACCCC	ACTTACCCTC	GATCGGACCA	CCACGCCGCG
8801				CTAGCCTGGT	
	GGCAGACCGA	CGAGCICCCC	TCANTGCCAC	CIAGCCIGGI	001000000
			0000000000	CGGTCGGAGC	TTC ATCACAA
8851					
	GCTCGGGTTT	CAGGTCTACA	GGCGCGCGCC	GCCAGCCTCG	AACTACTGTT
8901				GGAGCTCCCG	
	GTAGCGCGTC	TACCCTCGAC	AGGTACCAGA	CCTCGAGGGC	GCCGCAGTCC
8951				CATAGACGGG	
	AGTCCGCCCT	CGAGGACGTC	CAAATGGAGC	GTATCTGCCC	AGTCCCGCGC
9001	GGCTAGATCC	AGGTGATACC	TAATTTCCAG	GGGCTGGTTG	GTGGCGGCGT
3002				CCCGACCAAC	
	CCGATCIAGO				
9051	·CCATCCCTTC	ראאמאמממממ	CATCCCCCC	GCGCGACTAC	GGTACCGCGC
3031				CGCGCTGATG	
	GCIACCGAAC	GIICICCOGC	GIAGGGGGG		
		000000000		GATGATGCAT	CTAAAACCCC
9101					
	CCGCCCGCCA	CCCGGCGCCC	CCACAGGAAC	CTACTACGTA	GATTTICGCC
					0000000000
9151	TGACGCGGGC	GAGCCCCCGG	AGGTAGGGG	GGCTCCGGAC	CCGCCGGGAG
	ACTGCGCCCG	CTCGGGGGCC	TCCATCCCCC	CCGAGGCCTG	GGCGGCCCTC
				•	
9201	AGGGGGCAGG	GGCACGTCGG	CGCCGCGCGC	GGGCAGGAGC	TGGTGCTGCG
	TCCCCCGTCC	CCGTGCAGCC	GCGGCGCGCG	CCCGTCCTCG	ACCACGACGC
				•	
9251	CGCGTAGGTT	GCTGGCGAAC	GCGACGACGC	GGCGGTTGAT	CTCCTGAATC
	GCGCATCCAA	CGACCGCTTG	CGCTGCTGCG	CCGCCAACTA	GAGGACTTAG
	••••				
9301	ጥርር ርር ርር ርጥር ጥ	GCGTGAAGAC	GACGGGCCCG	GTGAGCTTGA	ACCTGAAAGA
9301	YCCCCCCTCT	CCCVCAACAC	כוופכיניניניני	CACTCGAACT	TGGACTTTCT
	ACCUCUUMUM	COCACIACIO			
0254	as ammaas as	ጣ እ ያመረግ እ የመጠ		ה מ≱רפטרפטרר	TGGCGCAAAA
3321	GAGT-TUGACA	GWW1CWW111	CCCVCVCVCVV	CACCOCCOCC	ACCGCGTTTT
	CTCAAGCTGT	CTTAGTTAAA	GCCACAGCAA	~13CCGCCGG	ACCOCG1111
				100001mom	CCC0380330
9401	TCTCCTGCAC	GTCTCCTGAG	TTGTCTTGAT	AGGUGATUTU	GGCCATGAAC
	AGAGGACGTG	CAGAGGACTC	AACAGAACTA	TCCGCTAGAG	CCGGTACTTG

Figure 26. J 64/144

9501			TGCGGGCCAT ACGCCCGGTA		
0551			CGGCTGTAGA		
9551					
	CCGGAGGGAG	CAAGGTCTGC	GCCGACATCT	GGTGCGGGGG	AAGCCGTAGC
9601	CGGGCGCGCA	TGACCACCTG	CGCGAGATTG	AGCTCCACGT	GCCGGGCGAA
	GCCCGCGCGT	ACTGGTGGAC	GCGCTCTAAC	TCGAGGTGCA	CGGCCCGCTT
9651	CACGGCGTAG	TTTCGCAGGC	GCTGAAAGAG	GTAGTTGAGG	GTGGTGGCGG
3031			CGACTTTCTC		
9701			TACATAACCC		
			ATGTATTGGG		
9751	TTGATATCCC	CCAAGGCCTC	AAGGCGCTCC	ATGGCCTCGT	AGAAGTCCAC
	AACTATAGGG	GGTTCCGGAG	TTCCGCGAGG	TACCGGAGCA	TCTTCAGGTG
9801	CCCGAACTTG	AAAAACTGGG	AGTTGCGCGC	CGACACGGTT	AACTCCTCCT
3001			TCAACGCGCG		
9851	• • •		GCGACAGTGT		
			CGCTGTCACA		
9901	GCTACAGGGG	CCTCTTCTTC	TTCTTCAATC	TCCTCTTCCA	TAAGGGCCTC
	CGATGTCCCC	GGAGAAGAAG	AAGAAGTTAG	AGGAGAAGGT	ATTCCCGGAG
9951	CCCTTCTTCT	TCTTCTGGCG	GCGGTGGGG	AGGGGGGACA	CGGCGGCGAC
			CGCCACCCC		
			TCGACAAAGC		
10001	-				
			AGCTGTTTCG		
10051			GACGGCGCGG		
	GCTGCCGCGT	ACCAGAGCCA	CTGCCGCGCC	GGCAAGAGCG	CCCCCGCGTC
10101	TTCGAAGACG	CCGCCCGTCA	TGTCCCGGTT	ATGGGTTGGC	GGGGGGCTGC
			ACAGGGCCAA		
10151	CATGCGGCAG				
10151			GATTGCTACG		
10201	GGTACTCCGC	CGCCGAGGGA	CCTGAGCGAG	TCCGCATCGA	CCGGATCGGA
	CCATGAGGCG	GCGGCTCCCT	GGACTCGCTC	AGGCGTAGCT	GGCCTAGCCT
10251	AAACCTCTCG	AGAAAGGCGT	CTAACCAGTC	ACAGTCGCAA	GGTAGGCTGA
			GATTGGTCAG		
				•	
10301	GCACCGTGGC				
			CCCGCCGCCA		
10351					GGCGGATGGT
	CACGACGACT	ACTACATTAA	TTTCATCCGC	CAGAACTCTG	CCGCCTACCA

Figure 26 K

10451	CGGCCATGCC	CCAGGCTTCG	TTTTGACATC	GGCGCAGGTC	TTTGTAGTAG
,		GGTCCGAAGC			
	GCCGGTACGG	001000.1.00			, p p 10, 11 C / 11 C
10501		GCCTTTCTAC	CCCC3 CMMCM	mcmacmccma	COMOMMONOC
10501					
	AGAACGTACT	CGGAAAGATG	GCCGTGAAGA	AGAAGAGGAA	GGAGAACAGG
10551	TGCATCTCTT	GCATCTATCG	CTGCGGCGGC	GGCGGAGTTT	GGCCGTAGGT
	ACGTAGAGAA	CGTAGATAGC	GACGCCGCCG	CCGCCTCAAA	CCGGCATCCA
					• • • • • • • • • • • • • • • • • • • •
10601	CCCCCCCCCC	TCCTCCCATG	CCTCTCACCC	CCAACCCCCT	CATCCCCTCA
10001					
	CCGCGGGAGA	AGGAGGGTAC	GCACACTGGG	GCTTCGGGGA	GIAGCCGACI
10651	AGCAGGGCTA	GGTCGGCGAC	AACGCGCTCG	GCTAATATGG	CCTGCTGCAC
	TCGTCCCGAT	CCAGCCGCTG	TTGCGCGAGC	CGATTATACC	GGACGACGTG
10701	CTGCGTGAGG	GTAGACTGGA	AGTCATCCAT	GTCCACAAAG	CGGTGGTATG
	GACGCACTCC				
	. uncocherce	CAICIGACCI	1030130013		000
		0. mcomom	OMOGN COMOGO	CC>D>>CCC>	CC2 CDD2 2 CC
10751		GATGGTGTAA			
	GCGGGCACAA	CTACCACATT	CACGTCAACC	GGTATTGCCT	GGTCAATTGC
	•				
10801	GTCTGGTGAC	CCGGCTGCGA	GAGCTCGGTG	TACCTGAGAC	GCGAGTAAGC
	CAGACCACTG	GGCCGACGCT	CTCGAGCCAC	ATGGACTCTG	CGCTCATTCG
					•
10851	CCTCGAGTCA	AATACGTAGT	CGTTGCAAGT	CCGCACCAGG	TACTGGTATC
10001		TTATGCATCA			
	GGAGCICAGI	TIMIGENIEN	GCAACGIICA	0000100100	Midnechino
					0000000000
10901		GTGCGGCGGC			
	GGTGGTTTTT	CACGCCGCCG	CCGACCGCCA	TCTCCCCGGT	CGCATCCCAC
10951	GCCGGGGCTC	CGGGGGCGAG	ATCTTCCAAC	ATAAGGCGAT	GATATCCGTA
	CGGCCCCGAG	GCCCCCGCTC	TAGAAGGTTG	TATTCCGCTA	CTATAGGCAT
11001	GATGTACCTG	GACATCCAGG	TGATGCCGGC	GCCGGTGGTG	GAGGCGCGCG
11001		CTGTAGGTCC			
	CINCAIGGAC	CIGIAGGICC	ACIACOGCCO	CCOCCACCAC	C10000000
		01.000000000	G > G > MOMMOO	002000022	N N N CMCCMCC
11051	GAAAGTCGCG				
	CTTTCAGCGC	CTGCGCCAAG	GTCTACAACG	CGTCGCCGTT	TTTCACGAGG
11101	ATGGTCGGGA				
	TACCAGCCCT	GCGAGACCGG	CCAGTCCGCG	CGCGTTAGCA	ACTGCGAGAT
		•			
11151	GACCGTGCAA	AAGGAGAGCC	TGTAAGCGGG	CACTCTTCCG	TGGTCTGGTG
11131		TTCCTCTCGG			
	CIGGCACGII	110010100	ACATTCOCCC	G1 GAGARIGGC	neer oncore
		0110000100	> manage = 2	1000000000	CX-CCCCCCCC
11201	GATAAATTCG				
	CTATTTAAGC	GTTCCCATAG	TACCGCCTGC	TGGCCCCAAG	CTCGGGGCAT
11251	TCCGGCCGTC	CGCCGTGATC	CATGCGGTTA	CCGCCCGCGT	GTCGAACCCA
		GCGGCACTAG			
			-		
11201	GGTGTGCGAC	COCACACAAC	הניניני <i>צ</i> יניזעריר	₼₼	ጥተርር ተመተርር ልር
11201		CAGTCTGTTG			
	CUACACGCTG	CAGICIGIIG	CCCCTCACG	MOGMMACCG	ANGGANGG IC

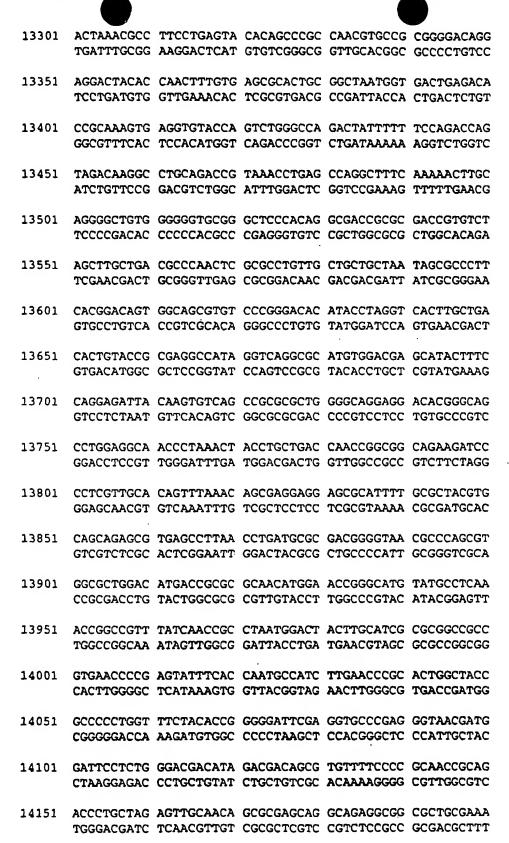
Figure 26L

11401	AAGCGGTTAG	GCTGGAAAGC	GAAAGCATTA	AGTGGCTCGC	TCCCTGTAGC
		CGACCTTTCG			
	licoccanic	COACCITICO	CITICOIMI	reneconoco	noooncareo
11451		TTTTCCAAGG			
	GCCTCCCAAT	AAAAGGTTCC	CAACTCAGCG	CCCTGGGGGC	CAAGCTCAGA
11501	CGGACCGGCC	GGACTGCGGC	GAACGGGGGT	TTGCCTCCCC	GTCATGCAAG
		CCTGACGCCG			
	000.00000				
	> 00000000000	CARAMMOOMO	0001110100	CACCACCCC	
11551		CAAATTCCTC			
	TGGGGCGAAC	GTTTAAGGAG	GCCTTTGTCC	CTGCTCGGGG	AAAAAACGAA
		•	•		
11601	TTCCCAGATG	CATCCGGTGC	TGCGGCAGAT	GCGCCCCCT	CCTCAGCAGC
	AAGGGTCTAC	GTAGGCCACG	ACGCCGTCTA	CGCGGGGGA	GGAGTCGTCG
11651	CCCAAGAGCA	AGAGCAGCGG	CAGACATGCA	GGGCACCCTC	CCCTCCTCCT
11001	-	TCTCGTCGCC			
	CCGIICICGI	icicolcocc	GICIGIACGI	cccaragana	GGGAGGAGGA
					a. a. maana.
11701		GAGGGGCGAC			
	TGGCGCAGTC	CTCCCCGCTG	TAGGCGCCAA	CTGCGCCGTC	GTCTACCACT
11751	TTACGAACCC	CCGCGGCGCC	GGGCCCGGCA	CTACCTGGAC	TTGGAGGAGG
	AATGCTTGGG	GGCGCCGCGG	CCCGGGCCGT	GATGGACCTG	AACCTCCTCC
11001	CCC N CCCCCC	GGCGCGGCTA	CCACCCCCCC	CTCCTCAGCG	CCACCCAAGG
11801					
	CGCTCCCGGA	CCGCGCCGAT	CCTCGCGGGA	GAGGACTCGC	CGIGGGIICC
11851		AGCGTGATAC			
	CACGTCGACT	TCGCACTATG	CGCACTCCGC	ATGCACGGCG	CCGTCTTGGA
11901	GTTTCGCGAC	CGCGAGGGAG	AGGAGCCCGA	GGAGATGCGG	GATCGAAAGT
		GCGCTCCCTC			
	0,22,0000,10	000010010			• • • • • • • • • • • • • • • • • • • •
11051	maa	GCGCGAGCTG	COCCARCOCC	MC N N MC C C C N	CCCCMACCAC
11951					
	AGGTGCGTCC	CGCGCTCGAC	GCCGTACCGG	ACT TAGCGCT	CGCCAACGAC
	•				
12001		ACTTTGAGCC			
•	GCGCTCCTCC	TGAAACTCGG	GCTGCGCGCT	TGGCCCTAAT	CAGGGCGCGC
12051	CGCACACGTG	GCGGCCGCCG	ACCTGGTAAC	CGCATACGAG	CAGACGGTGA
12031		Cecceccec			
	GCGIGIGCAC	000000000	100ACCA110	ocommoc.c	0.0.000
				>G>>GC>CC	CCCM3 CCCMT
12101	ACCAGGAGAT				
	TGGTCCTCTA	ATTGAAAGTT	TTTTCGAAAT	TGTTGGTGCA	CGCATGCGAA
12151	GTGGCGCGCG				
	CACCGCGCGC	TCCTCCACCG	ATATCCTGAC	TACGTAGACA	CCCTGAAACA
12201	AAGCGCGCTG	CACCAAAACC	СВВВТВССВВ	GCCGCTCATG	GCGCAGCTGT
12201					CGCGTCGACA
	TTUGUGUGAU	CICGIIIIGG	GITIMICGIT	COCCOMOTAC	COCOTCONCA
					2015
12251					GGATGCGCTG
	AGGAATATCA	CGTCGTGTCG	TCCCTGTTGC	TCCGTAAGTC	CCTACGCGAC

7 igure 26 M

12351		ATAGTGGTGC TATCACCACG			
12401		CAACTATTCC GTTGATAAGG			
12451	AAGATATACC	ATACCCCTTA	CGTTCCCATA	GACAAGGAGG	TAAAGATCGA
		TATGGGGAAT			
12501		ATGCGCATGG TACGCGTACC			
12551		TCGCAACGAG AGCGTTGCTC			
12601	CGGCGCGAGC GCCGCGCTCG	TCAGCGACCG AGTCGCTGGC	CGAGCTGATG GCTCGACTAC	CACAGCCTGC GTGTCGGACG	AAAGGGCCCT TTTCCCGGGA
12651		GGCAGCGGCG CCGTCGCCGC			
12701		GCGCTGGGCC			
12751	GCCGGACCTG	GGCTGGCGGT	GGCACCCGCG	CGCGCTGGCA	ACGTCGGCGG
12801		CCGACCGCCA			
12001	GCACCTCCTT	ATACTGCTCC	TGCTACTCAT	GCTCGGTCTC	CTGCCGCTCA
12851	ACTAAGCGGT TGATTCGCCA	GATGTTTCTG CTACAAAGAC	ATCAGATGAT TAGTCTACTA	GCAAGACGCA CGTTCTGCGT	ACGGACCCGG TGCCTGGGCC
12901	CGGTGCGGGC GCCACGCCCG	GGCGCTGCAG CCGCGACGTC	AGCCAGCCGT TCGGTCGGCA	CCGGCCTTAA GGCCGGAATT	CTCCACGGAC GAGGTGCCTG
12951	GACTGGCGCC GGCGCCAGTC	AGGTCATGGA TCCAGTACCT	CCGCATCATG GGCGTAGTAC	TCGCTGACTG AGCGACTGAC	CGCGCAATCC GCGCGTTAGG
13001	TGACGCGTTC ACTGCGCAAG	CGGCAGCAGC GCCGTCGTCG	CGCAGGCCAA GCGTCCGGTT	CCGGCTCTCC GGCCGAGAGG	GCAATTCTGG CGTTAAGACC
13051	AAGCGGTGGT TTCGCCACCA	CCCGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC	GCAAACCCCA CGTTTGGGGT	CGCACGAGAA GCGTGCTCTT	GGTGCTGGCG
13101	ATCGTAAACG TAGCATTTGC	CGCTGGCCGA	AAACAGGGCC TTTGTCCCGG	ATCCGGCCCG TAGGCCGGGC	ACGAGGCCGG TGCTCCGGCC
13151	CCTGGTCTAC GGACCAGATG	GACGCGCTGC CTGCGCGACG	TTCAGCGCGT AAGTCGCGCA	GGCTCGTTAC CCGAGCAATG	AACAGCGGCA TTGTCGCCGT
13201	ACGTGCAGAC	CAACCTGGAC	CGGCTGGTGG	GGGATGTGCG	CGAGGCCGTG GCTCCGGCAC

Figure 26 N



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14251					
74777	CCCGCTCAGA	TGCTAGTAGC	CCATTTCCAA	GCTTGATAGG	GTCTCTTACC
		ACGATCATCG			
	GCGCCAGTCT	ACGAICAICG	GGIAAAGGII	CONNCINICC	CAGAGAATGG
14301		CCACCGCCC			
	TCGTGAGCGT	GGTGGGCGGG	CGCGGACGAC	CCGCTCCTCC	TCATGGATTT
14351	CAACTCGCTG	CTGCAGCCGC	AGCGCGAAAA	AAACCTGCCT	CCGGCATTTC
14551		GACGTCGGCG			
	GIIONGCONC	4			
		GATAGAGAGC	CMACMCCACA	ACATCACTAC	ATGGAAGACG
14401	CCAACAACGG	GATAGAGAGC	CIAGIGGACA	AGAIGAGIAG	MA COMMONCO
	GGTTGTTGCC	CTATCTCTCG	GATCACCTGT	TCTACTCATC	TACCITCIGC
14451		AGCACAGGGA			
	ATGCGCGTCC	TCGTGTCCCT	GCACGGTCCG	GCCCCGGCC	GGTGGGCAGC
14501	TCAAAGGCAC	GACCGTCAGC	GGGGTCTGGT	GTGGGAGGAC	GATGACTCGG
		CTGGCAGTCG			
	AGITICCOIC	c	0000		
. 4553	an an accordance	CAGCGTCCTG	CATTTCCCAC	CCACTCCCAA	ССССТТТССС
14551		GTCGCAGGAC			
	GTCTGCTGTC	GTCGCAGGAC	CTAAACCCTC	CCICACCGII	GGGCAAACGC
14601		CCAGGCTGGG			
	GTGGAAGCGG	GGTCCGACCC	CTCTTACAAA	ATTTTTTTT	TTTTCGTACT
14651		AAAACTCACC			
	ACGTTTTATT	TTTTGAGTGG	TTCCGGTACC	GTGGCTCGCA	ACCAAAAGAA
14701	CTATTCCCCT	TAGTATGCGG	CGCGCGGCGA	TGTATGAGGA	AGGTCCTCCT
14,01	CATAACCCCA	ATCATACGCC	CCCCCCCCC	ACATACTCCT	TCCAGGAGGA
	CATANGGGGA	AICAIACOCC	ocococicoe:		
		> a> amamaan	CACCCCCCC	CCACTCCCCC	CCCCCCTCCC
	~~~~~~~~~~~				
14751	CCCTCCTACG	AGAGTGTGGT	0700000000	CCMCACCCCC	CCCCCACCC
14751	CCCTCCTACG GGGAGGATGC	TCTCACACCA	CTCGCGCCGC	GGTCACCGCC	GCCGCGACCC
	GGGAGGATGC	TCTCACACCA	CTCGCGCCGC	GGTCACCGCC	GCCGCGACCC
14751	GGGAGGATGC TTCTCCCTTC	TCTCACACCA GATGCTCCCC	CTCGCGCCGC TGGACCCGCC	GGTCACCGCC GTTTGTGCCT	GCCGCGACCC CCGCGGTACC
	GGGAGGATGC TTCTCCCTTC	TCTCACACCA	CTCGCGCCGC TGGACCCGCC	GGTCACCGCC GTTTGTGCCT	GCCGCGACCC CCGCGGTACC
	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG	TCTCACACCA GATGCTCCCC CTACGAGGGG	CTCGCGCCGC TGGACCCGCC ACCTGGGCGG	GGTCACCGCC GTTTGTGCCT CAAACACGGA	GCCGCGACCC CCGCGGTACC GGCGCCATGG
	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG	TCTCACACCA GATGCTCCCC CTACGAGGGG	CTCGCGCCGC TGGACCCGCC ACCTGGGCGG	GGTCACCGCC GTTTGTGCCT CAAACACGGA	GCCGCGACCC CCGCGGTACC GGCGCCATGG
14801	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG TGCGGCCTAC	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGAGA	TGGACCCGCC ACCTGGGCGG AACAGCATCC	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC
14801	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG TGCGGCCTAC	TCTCACACCA GATGCTCCCC CTACGAGGGG	TGGACCCGCC ACCTGGGCGG AACAGCATCC	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC
14801	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG TGCGGCCTAC ACGCCGGATG	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT	TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG
14801	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG TGCGGCCTAC ACGCCGGATG CTATTCGACA	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT CCACCCGTGT	TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACGGATGT
14801	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG TGCGGCCTAC ACGCCGGATG CTATTCGACA	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT CCACCCGTGT	TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG
14801 14851 14901	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG TGCGGCCTAC ACGCCGGATG CTATTCGACA GATAAGCTGT	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCTCT CCACCCGTGT GGTGGGCACA	TGGACCGCC TGGACCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACGGATGT GTTGCCTACA
14801 14851 14901	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG TGCGGCCTAC ACGCCGGATG CTATTCGACA GATAAGCTGT GGCATCCCTG	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA	TTGGCGCCGC TGGACCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC ACGACCACAG	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACGGATGT GTTGCCTACA ACCACGGTCA
14801 14851 14901	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG TGCGGCCTAC ACGCCGGATG CTATTCGACA GATAAGCTGT GGCATCCCTG	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA	TTGGCGCCGC TGGACCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC ACGACCACAG	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACGGATGT GTTGCCTACA
14801 14851 14901 14951	GGGAGGATGC TTCTCCCTTC AAGAGGGAAG TGCGGCCTAC ACGCCGGATG CTATTCGACA GATAAGCTGT GGCATCCCTG CCGTAGGGAC	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA TTGATGGTCT	TGGACCCGCC TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCACAC ACGACCACAG TGCTGGTGTC	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG GTTGAAAGAC	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACCGTGGT GTTGCCTACA ACCACGGTCA TGGTGCCAGT
14801 14851 14901 14951	GGGAGGATGC  TTCTCCCTTC AAGAGGGAAG  TGCGGCCTAC ACGCCGGATG  CTATTCGACA GATAAGCTGT  GGCATCCCTG CCGTAGGGAC	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA TTGATGGTCT TGACTACAGC	TGGACCCGC TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC ACGACCACAG TGCTGGTGTC CCGGGGGAGG	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG GTTGAAAGAC CAAGCACACA	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACCGTGGT GTTGCCTACA ACCACGGTCA TGGTGCCAGT
14801 14851 14901 14951	GGGAGGATGC  TTCTCCCTTC AAGAGGGAAG  TGCGGCCTAC ACGCCGGATG  CTATTCGACA GATAAGCTGT  GGCATCCCTG CCGTAGGGAC	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA TTGATGGTCT TGACTACAGC	TGGACCCGC TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC ACGACCACAG TGCTGGTGTC CCGGGGGAGG	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG GTTGAAAGAC CAAGCACACA	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACCGTGGT GTTGCCTACA ACCACGGTCA TGGTGCCAGT
14801 14851 14901 14951 15001	GGGAGGATGC  TTCTCCCTTC AAGAGGGAAG  TGCGGCCTAC ACGCCGGATG  CTATTCGACA GATAAGCTGT  GGCATCCCTG CCGTAGGGAC  TTCAAAACAA AAGTTTTGTT	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA TTGATGCTCT TGACTACAGC ACTGATGTCG	TGGACCCGCC TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC ACGACCACAG TGCTGGTGTC CCGGGGGAGG GGCCCCTCC	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG GTTGAAAGAC CAAGCACACA GTTCGTGTGT	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACGGATGT GTTGCCTACA ACCACGGTCA TGGTGCCAGT GACCATCAAT CTGGTAGTTA
14801 14851 14901 14951 15001	GGGAGGATGC  TTCTCCCTTC AAGAGGGAAG  TGCGGCCTAC ACGCCGGATG  CTATTCGACA GATAAGCTGT  GGCATCCCTG CCGTAGGGAC  TTCAAAACAA AAGTTTTGTT	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA TTGATGCTCT TGACTACAGC ACTGATGTCG	TGGACCCGC TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC ACGACCACAG TGCTGGTGTC CCGGGGGAGG GGCCCCTCC	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG GTTGAAAGAC CTGTGTGTGT CTGAAAACCA	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACGGATGT GTTGCCTACA ACCACGGTCA TGGTGCCAGT GACCATCAAT CTGGTAGTTA
14801 14851 14901 14951 15001	GGGAGGATGC  TTCTCCCTTC AAGAGGGAAG  TGCGGCCTAC ACGCCGGATG  CTATTCGACA GATAAGCTGT  GGCATCCCTG CCGTAGGGAC  TTCAAAACAA AAGTTTTGTT	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA TTGATGCTCT TGACTACAGC ACTGATGTCG	TGGACCCGC TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC ACGACCACAG TGCTGGTGTC CCGGGGGAGG GGCCCCTCC	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG GTTGAAAGAC CTGTGTGTGT CTGAAAACCA	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACGGATGT GTTGCCTACA ACCACGGTCA TGGTGCCAGT GACCATCAAT CTGGTAGTTA
14801 14851 14901 14951 15001	GGGAGGATGC  TTCTCCCTTC AAGAGGGAAG  TGCGGCCTAC ACGCCGGATG  CTATTCGACA GATAAGCTGT  GGCATCCCTG CCGTAGGGAC  TTCAAAACAA AAGTTTTGTT	TCTCACACCA GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA TTGATGCTCT TGACTACAGC ACTGATGTCG	TGGACCCGC TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC ACGACCACAG TGCTGGTGTC CCGGGGGAGG GGCCCCTCC	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG GTTGAAAGAC CTGTGTGTGT CTGAAAACCA	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACGGATGT GTTGCCTACA ACCACGGTCA TGGTGCCAGT GACCATCAAT CTGGTAGTTA
14801 14851 14901 14951 15001	GGGAGGATGC  TTCTCCCTTC AAGAGGGAAG  TGCGGCCTAC ACGCCGGATG  CTATTCGACA GATAAGCTGT  GGCATCCCTG CCGTAGGGAC  TTCAAAACAA AAGTTTTGTT  CTTGACGACC GAACTGCTGG	GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA TTGATGGTCT TGACTACAGC ACTGATGTCG GGTCGCACTG	TGGACCGCGC TGGACCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC ACGACCACAG TGCTGGTGTC CCGGGGGAGG GGCCCCCTCC GGGCGGCGAC CCCGCCGCTG	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG GTTGAAAGAC CAAGCACACA GTTCGTGTGT CTGAAAACCA GACTTTTGGT	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACGGATGT GTTGCCTACA ACCACGGTCA TGGTGCCAGT GACCATCAAT CTGGTAGTTA TCCTGCATAC AGGACGTATG
14801 14851 14901 14951 15001	GGGAGGATGC  TTCTCCCTTC AAGAGGGAAG  TGCGGCCTAC ACGCCGGATG  CTATTCGACA GATAAGCTGT  GGCATCCCTG CCGTAGGGAC  TTCAAAACAA AAGTTTTGTT  CTTGACGACC GAACTGCTG	GATGCTCCCC CTACGAGGGG CGGGGGGAGA GCCCCCTCT CCACCCGTGT GGTGGGCACA AACTACCAGA TTGATGGTCT TGACTACAGC ACTGATGTCG GGTCGCACTG CCAGCGTGAC	TTCGCGCCGC TGGACCCGCC ACCTGGGCGG AACAGCATCC TTGTCGTAGG GTACCTGGTG CATGGACCAC ACGACCACAG TGCTGGTGTC CCGGGGGAGG GGCCCCTCC GGGCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCCGCC	GGTCACCGCC GTTTGTGCCT CAAACACGGA GTTACTCTGA CAATGAGACT GACAACAAGT CTGTTGTTCA CAACTTTCTG GTTGAAAGAC CAAGCACACA GTTCGTGTGT CTGAAAACCA GACTTTTGGT TACCAATAAG	GCCGCGACCC CCGCGGTACC GGCGCCATGG GTTGGCACCC CAACCGTGGG CAACGGATGT GTTGCCTACA ACCACGGTCA TGGTGCCAGT GACCATCAAT CTGGTAGTTA

Figure 26 P

WO 02/022080					PCT/US01/28861
15151	CC TCCT	GTCGCGCTTG	CCTACTAAGG	ACAATCAG	GAGCTGAAA
13131		CAGCGCGAAC			
		TGGAGTTCAC ACCTCAAGTG		· · · · · · ·	
15251		CTTATGAACA GAATACTTGT			
15301		CGGGGTTCTG GCCCCAAGAC			
15351		GACTGGGGTT CTGACCCCAA			TCATGCCTGG AGTACGGACC
15401		AACGAAGCCT TTGCTTCGGA			
<b>154</b> 51		CTTCACCCAC GAAGTGGGTG			
. 15501		CCTTCCAGGA GGAAGGTCCT			
15551		ATTCCCGCAC TAAGGGCGTG		•	
15601		CACCGAACAG GTGGCTTGTC			
15651		GCGCGGAAGA CGCGCCTTCT			
15701		GACATGAACG CTGTACTTGC			
15751		GGAGAAGCGC CCTCTTCGCG			
15801		CGCAACCCGA GCGTTGGGCT			
15851		ACAGAGGACA TGTCTCCTGT			
15901		CTTCACCCAG GAAGTGGGTC			
15951		AGACCGGAAT TCTGGCCTTA			GCACTCCTGA CGTGAGGACT
16001		GGCTCGGAGC CCGAGCCTCG			
16051		GACCTTCCGC CTGGAAGGCG			

Figure 26 Q

WO 02/022080 PCT/US01/28861

16151	GGCCGTCTAC	TCCCAACTCA	TCCGCCAGTT	TACCTCTCTG	ACCCACGTGT
			AGGCGGTCAA		
	CC000				
		<b>******</b>	CAGATTTTGG		***************************************
16201					
	AGTTAGCGAA	AGGGCTCTTG	GTCTAAAACC	GCGCGGGCGG	TCGGGGGTGG
16251	ATCACCACCG	TCAGTGAAAA	CGTTCCTGCT	CTCACAGATC	ACGGGACGCT
			GCAAGGACGA		
	1401001000				
		>> 0> 00> maa	GAGGAGTCCA	CCCNCCCNCC	ATTACTCACC
16301					
	TGGCGACGCG	TTGTCGTAGC	CTCCTCAGGT	CGCTCACTGG	TAATGACTGC
16351			TACGTTTACA		
	GGTCTGCGGC	GTGGACGGGG	ATGCAAATGT	TCCGGGACCC	GTATCAGAGC
16401	CCCCCCCTCC	TATCGAGCCG	CACTTTTTGA	GCAAGCATGT	CCATCCTTAT
10401	000000000	AMACCINCCCC	GTGAAAAACT	ССТТССТАСА	CCTACCAATA
	GGCGCGCAGG	ATAGCTCGGC	GIGNAMACI	COLICGIACA	0011100111111
•					1001101000
16451			GCTGGGGCCT		
	TAGCGGGTCG	TTATTGTGTC	CGACCCCGGA	CGCGAAGGGT	TCGTTCTACA
16501	TTGGCGGGGC	CAAGAAGCGC	TCCGACCAAC	ACCCAGTGCG	CGTGCGCGGG
20302	AACCGCCCCG	GTTCTTCGCG	AGGCTGGTTG	TGGGTCACGC	GCACGCGCCC
	Miccoccco	021011000			
	a. am. aaaaa	6000000CCC	CGCGCACAAA	CCCCCCCCA	CTGGGCGCAC
16551	CACTACCGCG	CGCCCTGGGG	CGCGCACAAA	COCCOCCA	CACCOCCEC
	GTGATGGCGC	GCGGGACCCC	GCGCGTGTTT	GCGCCGGCG1	GACCCGCGIG
16601					CGCAACTACA
	GTGGCAGCTA	CTGCGGTAGC	TGCGCCACCA	CCTCCTCCGC	GCGTTGATGT
			•		
16651	CCCCCACGCC	GCCACCAGTG	TCCACAGTGG	ACGCGGCCAT	TCAGACCGTG
10031			AGGTGTCACC		
	GCGGG1GCGG	CGGIGGICAC	Addidicace	100000001	
			maamaaaa	**********	CCACCCCCCT
16701					GGAGGCGCGT
	CACGCGCCTC	GGGCCGCGAT	ACGATTTTAC	TTCTCTGCCG	CCTCCGCGCA
16751	AGCACGTCGC	CACCGCCGCC	GACCCGGCAC	TGCCGCCCAA	CGCGCGGCGG
					GCGCGCCGCC
		************			
1.0001	000000mccm	ma a coccoco	CCTCCCACCC	GCCGACGGGC	GGCCATGCGG
10801	CGGCCCTGCT	177666666	COLCOCACCO	CCCCTCCCC	CCGGTACGCC
	GCCGGGACGA	ATTGGCGCGT	GCAGCG1GGC	COOCTOCCCO	CCGGIACGCC
					001000000
16851	GCCGCTCGAA	GGCTGGCCGC	GGGTATTGTC	ACTGTGCCCC	CCAGGTCCAG
	CGGCGAGCTT	CCGACCGGCG	CCCATAACAG	TGACACGGGG	GGTCCAGGTC
					•
16901	GCGACGAGCG	GCCGCCGCAG	CAGCCGCGGC	CATTAGTGCT	ATGACTCAGG
20701	CCCACCACC	CGGCGGCGAC	GTCGGCGCCC	GTAATCACGA	TACTGAGTCC
	CGC1GC1CGC	2990990910			
		011000000	manana an	PCMCCCWub C	רפפריזיפיפי
16951	GTCGCAGGGG	CAACGIGIAI	100010000	WC 1 CGG 1 1WG	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
	CAGCGTCCCC	GTTGCACATA	ACCCACGCGC	TGAGCCAATC	GCCGGACGCG
17001	GTGCCCGTGC	GCACCCGCCC	CCCGCGCAAC	TAGATTGCAA	GAAAAAACTA
	CACGGGCACG	CGTGGGCGGG	GGGCGCGTTG	ATCTAACGTT	CTTTTTTGAT

7igure 26 R

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PCT/US01/28861

17101 CTATGT:CAA GCGCAAAATC AAAGAAGAGA TGCTCCAGGT CATCGCGCCG
GATACAGGTT CGCGTTTTAG TTTCTTCTCT ACGAGGTCCA GTAGCGCGGC

17101			AAAGAAGAGA TTTCTTCTCT		
17151			GAAGGAAGAG CTTCCTTCTC		
	CICIAGAIAC	C666666C11	CITCCITCIC	GICCIANIGI	1000000111
17201			AAAAGAAAGA		
			TTTTCTTTCT		
17251			GCTACCGCGC		
			CGATGGCGCG		•
17301			TGTTTTGCGA		
			ACAAAACGCT		
17351			CCCGCACCTA		
			GGGCGTGGAT		
17401			CTTGAGCAGG		
			GAACTCGTCC		
17451			TAAGGACATG		
	AAACGGATGC	CTTTCGCCGT	ATTCCTGTAC	GACCGCAACG	GCGACCTGCT
17501			TAAAGCCCGT		
			ATTTCGGGCA		
17551			GAAAAGCGCG		
		_	CTTTTCGCGC		
17601	GACTTGGCAC	CCACCGTGCA	GCTGATGGTA	CCCAAGCGCC	AGCGACTGGA
			CGACTACCAT	,	
17651			CCGTGGAACC		
			GGCACCTTGG		
17701			GTGGCGCCGG		
			CACCGCGGCC		
17751	GACGTTCAGA				
			GTCATCGTGG		
17801	GGGCATGGAG				
	CCCGTACCTC	TGTGTTTGCA	GGGGCCAACG	GAGTCGCCAC	CGCCTACGGC
17851	CGGTGCAGGC				
	GCCACGTCCG	CCAGCGACGC	CGGCGCAGGT	TCTGGAGATG	CCTCCACGTT
17901	ACGGACCCGT				
	TGCCTGGGCA	CCTACAAAGC	GCAAAGTCGG	GGGGCCGCGG	GCGCGGCAAG
17951	GAGGAAGTAC				
	CTCCTTCATG	CCGCGGCGGT	CGCGCGATGA	CGGGCTTATA	CGGGATGTAG

Figure 265

18051		CTACCCGACG			
	TCTGCTCGTT	GATGGGCTGC	GGCTTGGTGG	TGACCTTGGG	CGGCGGCGGC
18101		CAGCCCGTGC			
	AGCGGCAGCG	GTCGGGCACG	ACCGGGGCTA	AAGGCACGCG	TCCCACCGAG
18151	GCGAAGGAGG	CAGGACCCTG	GTGCTGCCAA	CAGCGCGCTA	CCACCCCAGC
		GTCCTGGGAC			
18201	ATCGTTTAAA	AGCCGGTCTT	TGTGGTTCTT	GCAGATATGG	CCCTCACCTG
	TAGCAAATTT	TCGGCCAGAA	ACACCAAGAA	CGTCTATACC	GGGAGTGGAC
18251		TTCCCGGTGC			
	GGCGGAGGCA	AAGGGCCACG	GCCCTAAGGC	TCCTTCTTAC	GTGGCATCCT
18301	GGGGCATGGC				
		GCCGGTGCCG			
18351		GCGCGTCGCA			
		CGCGCAGCGT			
18401		CTGATCGCCG			
		GACTAGCGGC			
18451		GCAGGCGCAG			
		CGTCCGCGTC			
18501		ATAAAAAGTC			
		TATTTTTCAG			
18551		AATGGAAGAC			
		TTACCTTCTG			
18601		CGTTCATGGG			
		GCAAGTACCC			
18651		GCCTTCAGCT			
•		CGGAAGTCGA			
18701					CAGCAGCACA
					GTCGTCGTGT
18751	GGCCAGATGC CCGGTCTACG	TGAGGGATAA ACTCCCTATT	GTTGAAAGAG CAACTTTCTC	CAAAATTTCC GTTTTAAAGG	AACAAAAGGT TTGTTTTCCA
				~~~~~~	0000001100
18801	GGTAGATGGC	CTGGCCTCTG	GCATTAGCGG	GGTGGTGGAC	CTGGCCAACC
	•		•		GACCGGTTGG
18851					CCCTCCCGTA
					GGGAGGGCAT
18901	GAGGAGCCTC	CACCGGCCGT	GGAGACAGTG	TCTCCAGAGG	GGCGTGGCGA
	CTCCTCGGAG	GTGGCCGGCA	CCTCTGTCAC	AGAGGTCTCC	CCGCACCGCT

Figure 26T

PCT/US01/28861

19001	AGCCTCCCTC	GTACGAGGAG	GCACTAAAGC	AAGGCCTGCC	CACCACCCGT
				TTCCGGACGG	
	1CGGNGGGNG	CHIGGICCIC	CGIGATIICO	110000000	010010000
19051				GGCCAGCACA	
	GGGTAGCGCG	GGTACCGATG	GCCTCACGAC	CCGGTCGTGT	GTGGGCATTG
	••••				
19101				GCAGAAACCT	
	CGACCTGGAC	GGAGGGGGGC	GGCTGTGGGT	CGTCTTTGGA	CACGACGGTC
19151	CCCCCACCCC	CCTTCTTCTA	A C C C C T C C T A	GCCGCGCGTC	רכזיפרפרכפר
19131					
	CGGGCTGGCG	GCAACAACAT	TGGGCAGGAT	CGGCGCGCAG	GGALGLGGLG
				•	
19201	GCCGCCAGCG	GTCCGCGATC	GTTGCGGCCC	GTAGCCAGTG	GCAACTGGCA
	CGGCGGTCGC	CAGGCGCTAG	CAACGCCGGG	CATCGGTCAC	CGTTGACCGT
				• • • • • • • • • • • • • • • • • • • •	
	>> CC> C> CDC	>> c> cc> maa	mcccmcmccc	GGTGCAATCC	כתיכא א כככככ
19251					
	TTCGTGTGAC	TTGTCGTAGC	ACCCAGACCC	CCACGTTAGG	GACTTCGCGG
19301	GACGATGCTT	CTGATAGCTA	ACGTGTCGTA	TGTGTGTCAT	GTATGCGTCC
				ACACACAGTA	
	CIGCIACGAA	OACIMICOM!			
				000000000	CDDDCC AACA
19351				CGCGCGCCCG	
	TACAGCGGCG	GTCTCCTCGA	CGACTCGGCG	GCGCGCGGC	GAAAGGTTCT
19401	TGGCTACCCC	TTCGATGATG	CCGCAGTGGT	CTTACATGCA	CATCTCGGGC
				GAATGTACGT	
	ACCUATUGGG	MOCIACIAC	GGCGTCACCA	0.2000.	01002000
19451				CTGGTGCAGT	
	GTCCTGCGGA	GCCTCATGGA	CTCGGGGCCC	GACCACGTCA	AACGGGCGCG
19501	CACCGAGACG	TACTTCAGCC	TGAATAACAA	GTTTAGAAAC	CCCACGGTGG
10001				CAAATCTTTG	
	GIGGUILIGU	AIGAAGICGG	ACTIATION	CAAAICIIIG	GGGIGCCACC
		•			
19551				CCCAGCGTTT	
	GCGGATGCGT	GCTGCACTGG	TGTCTGGCCA	GGGTCGCAAA	CTGCGACGCC
19601	שייר א יירר כיירני	TOCACCOTCA	CCATACTCCC	TACTCGTACA	AGGCGCGGTT
19601					
	AAGTAGGGAC	ACCTGGCACT	CCTATGACGC	ATGAGCATGT	TCCGCCCAA
19651	CACCCTAGCT	GTGGGTGATA	ACCGTGTGCT	GGACATGGCT	TCCACGTACT
	GTGGGATCGA	CACCCACTAT	TGGCACACGA	CCTGTACCGA	AGGTGCATGA
	••••				
10201	mmax ax maga	CCCCCCCCCC	CACACCCCCC	ርጥ አርጥጥጥጥ አ	GCCCTACTCT
19/01					
	AACTGTAGGC	GCCGCACGAC	CTGTCCCCGG	GATGAAAATT	CGGGATGAGA
19751	GGCACTGCCT	ACAACGCCCT	GGCTCCCAAG	GGTGCCCCAA	ATCCTTGCGA
				CCACGGGGTT	
	CCG1 GACGGA	-0.100000			
		00mcca:::-:	0000000	******	CAACACCACC
19801					GAAGAGGACG
	TACCCTACTT	CGACGATGAC	GAGAACTTTA	TTTGGATCTT	CTTCTCCTGC
19851	ATGACAACGA	AGACGAAGTA	GACGAGCAAG	CTGAGCAGCA	AAAAACTCAC
					TTTTTGAGTG
	'WC1G11GC1	TOTACTION	2.00.00110		

Figure 26 U

19951	TCAAATAGGT	GTCGAAGGTC	AAACACCTAA	ATATGCCGAT	AAAACATTTC
		CAGCTTCCAG			
	AGILIATOR	C.1.001.100.10			
20001	>>00m0>>00	TCAAATAGGA	C	CCTACCAAAC	ДСДДДТТАДТ
20001					
	TTGGACTTGG	AGTTTATCCT	CTTAGAGTCA	CCATGCTTTG	TCTTTAATTA
20051		GGAGAGTCCT			
	GTACGTCGAC	CCTCTCAGGA	TTTTTTCTGA	TGGGGTTACT	TTGGTACAAT
20101	CGGTTCATAT	GCAAAACCCA	CAAATGAAAA	TGGAGGGCAA	GGCATTCTTG
	GCCAAGTATA	CGTTTTGGGT	GTTTACTTTT	ACCTCCCGTT	CCGTAAGAAC
		•			
20151	TANAGCAACA	AAATGGAAAG	CTAGAAAGTC	AAGTGGAAAT	GCAATTTTTC
		TTTACCTTTC			
•	Allicalia	111			
20201	mc > > cm > cmc	AGGCAGCCGC	ACCCAATGGT	САТААСТТСА	CTCCTAAAGT
20201		TCCGTCGGCG			
	AGTTGATGAC	TCCGTCGGCG	ICCGIIACCA	CIMITOMACI	GAGGAII I C
		AGTGAAGATG	m> C> m> m> C>	*******	አርጥር አጥአጥጥጥ
20251					
	CCATAACATG	TCACTTCTAC	ATCTATATCT	TIGGGGTCIG	IGNGINIAAA
		_			
20301		CACTATTAAG			
	GAATGTACGG	GTGATAATTC	CTTCCATTGA	GTGCTCTTGA	TTACCCGGTT
20351		CCAACAGGCC			
	GTTAGATACG	GGTTGTCCGG	ATTAATGTAA	CGAAAATCCC	TGTTAAAATA
20401	TGGTCTAATG	TATTACAACA	GCACGGGTAA	TATGGGTGTT	CTGGCGGGCC
	ACCAGATTAC	ATAATGTTGT	CGTGCCCATT	ATACCCACAA	GACCGCCCGG
20451	AAGCATCGCA	GTTGAATGCT	GTTGTAGATT	TGCAAGACAG	AAACACAGAG
		CAACTTACGA			
	.,	••••		•	
20501	CTTTC 1 T1CC	AGCTTTTGCT	TGATTCCATT	GGTGATAGAA	CCAGGTACTT
20301	GAAAGTATGG		ACTAAGGTAA		
	GAAAGIAIGG	1 COMMICON	AC1781001721	CC	
20553	mmom> momoc	AATCAGGCTG	中 型で か ご か ご ご で か か	TCATCCAGAT	CTTAGAATTA
20551		TTAGTCCGAC			
	AAGATACACC	TTAGTCCGAC	AACTGTCGAT	ACIAGGICIA	CARICIANA
			61 mc11 00mc	G3.3.5 MM3.0MC	CMMMCCACMC
20601	TTGAAAATCA	TGGAACTGAA	GATGAACTTC	CAAATTACTG	CITICCACIG
	AACTTTTAGT	ACCTTGACTT	CTACTTGAAG	GTTTAATGAC	GAAAGGTGAC
			_		
20651	GGÄGGTGTGA	TTAATACAGA	GACTCTTACC	AAGGTAAAAC	CTAAAACAGG
	CCTCCACACT	AATTATGTCT	CTGAGAATGG	TTCCATTTTG	GATTTTGTCC
20701	TCAGGAAAAT	GGATGGGAAA	AAGATGCTAC	AGAATTTTCA	GATAAAAATG
	AGTCCTTTTA	CCTACCCTTT	TTCTACGATG	TCTTAAAAGT	CTATTTTTAC
20751	AAATAAGAGT	TGGAAATAAT	TTTGCCATGG	AAATCAATCT	AAATGCCAAC
	TTTATTCTCA	ACCTTTATTA	AAACGGTACC	TTTAGTTAGA	TTTACGGTTG
20201	СТСТССАСАА	ልተተተርርጥርጥል	CTCCAACATA	GCGCTGTATT	TGCCCGACAA
20001	CIGIGGROUN	TARACCACAC	СУССТТСТУТ	CGCGACATAA	ACGGGCTGTT
	GWCWCC 1C11	*WYGGWCV*	J		

Tigure 26 V



20901	ACGACTACAT	GAACAAGCGA	GTGGTGGCTC	CCGGGCTAGT	GGACTGCTAC
	TGCTGATGTA	CTTGTTCGCT	CACCACCGAG	GGCCCGATCA	CCTGACGATG
20951		GAGCACGCTG			
	TAATTGGAAC	CTCGTGCGAC	CAGGGAACTG	ATATACCTGT	TGCAGTTGGG
21001		CACCGCAATG			
	TAAATTGGTG	GTGGCGTTAC	GACCGGACGC	GATGGCGAGT	TACAACGACC
21051		CTATGTGCCC			
		GATACACGGG			
21101		ACCTCCTTCT			,
		TGGAGGAAGA			
21151		GATGTTAACA			
		CTACAATTGT			
21201		CGGAGCCAGC			
	ATTCCCAACT	GCCTCGGTCG	TAATTCAAAC	TATCGTAAAC	GGAAATGUGG
21251		CCATGGCCCA			
	TGGAAGAAGG	GGTACCGGGT	GTTGTGGCGG	AGGTGCGAAC	TCCGGTACGA
21301		ACCAACGACC			
	ATCTTTGCTG	TGGTTGCTGG	TCAGGAAATT	GCTGATAGAG	AGGCGGCGGT
21351		CCCTATACCC			
		GGGATATGGG			
21401		ACTGGGCGGC			
	·	TGACCCGCCG			٠
21451		ACCCCATCAC			
•	CIGATICCTI	TGGGGTAGTG	ACCCGAGCCC	GATGCTGGGA	ATAATGTGGA
21501		TATACCCTAC	•	• • • • • • • • • •	
	TGAGACCGAG	ATATGGGATG	GATCTACCTT	GGAAAATGGA	GTTGGTGTGG
21551	TTTAAGAAGG				
	AAATTCTTCC	ACCGGTAATG	GAAACTGAGA	AGACAGTCGA	CCGGACCGTT
21601	TGACCGCCTG				
	ACTGGCGGAC	GAATGGGGGT	TGCTCAAACT	TTAATTCGCG	AGTCAACTGC
21651	GGGAGGGTTA				
	CCCTCCCAAT	GTTGCAACGG	GTCACATTGT	ACTGGTTTCT	GACCAAGGAC
21701	GTACAAATGC				
	CATGTTTACG	ATCGATTGAT	ATTGTAACCG	ATGGTCCCGA	AGATATAGGG
21751	AGAGAGCTAC				
	TCTCTCGATG	TTCCTGGCGT	ACATGAGGAA	GAAATCTTTG	AAGGTCGGGT

Figure 26 W

PCT/US01/28861 21851 GGCATECTAC ACCAACACAA CAACTCTGGA TTTGTTGGCT ACCTTGCCCC CCGTAGGATG TGGTTGTGTT GTTGAGACCT AAACAACCGA TGGAACGGGG 21901 CACCATGCGC GAAGGACAGG CCTACCCTGC TAACTTCCCC TATCCGCTTA GTGGTACGCG CTTCCTGTCC GGATGGGACG ATTGAAGGGG ATAGGCGAAT 21951 TAGGCAAGAC CGCAGTTGAC AGCATTACCC AGAAAAAGTT TCTTTGCGAT ATCCGTTCTG GCGTCAACTG TCGTAATGGG TCTTTTTCAA AGAAACGCTA 22001 CGCACCCTTT GGCGCATCCC ATTCTCCAGT AACTTTATGT CCATGGGCGC GCGTGGGAAA CCGCGTAGGG TAAGAGGTCA TTGAAATACA GGTACCCGCG 22051 ACTCACAGAC CTGGGCCAAA ACCTTCTCTA CGCCAACTCC GCCCACGCGC TGAGTGTCTG GACCCGGTTT TGGAAGAGAT GCGGTTGAGG CGGGTGCGCG 22101 TAGACATGAC TTTTGAGGTG GATCCCATGG ACGAGCCCAC CCTTCTTTAT ATCTGTACTG AAAACTCCAC CTAGGGTACC TGCTCGGGTG GGAAGAAATA 22151 GTTTTGTTTG AAGTCTTTGA CGTGGTCCGT GTGCACCAGC CGCACCGCGG CAAAACAAAC TTCAGAAACT GCACCAGGCA CACGTGGTCG GCGTGGCGCC 22201 CGTCATCGAA ACCGTGTACC TGCGCACGCC CTTCTCGGCC GGCAACGCCA GCAGTAGCTT TGGCACATGG ACGCGTGCGG GAAGAGCCGG CCGTTGCGGT 22251 CAACATAAAG AAGCAAGCAA CATCAACAAC AGCTGCCGCC ATGGGCTCCA GTTGTATTTC TTCGTTCGTT GTAGTTGTTG TCGACGGCGG TACCCGAGGT 22301 GTGAGCAGGA ACTGAAAGCC ATTGTCAAAG ATCTTGGTTG TGGGCCATAT CACTCGTCCT TGACTTTCGG TAACAGTTTC TAGAACCAAC ACCCGGTATA 22351 TTTTTGGGCA CCTATGACAA GCGCTTTCCA GGCTTTGTTT CTCCACACAA AAAAACCCGT GGATACTGTT CGCGAAAGGT CCGAAACAAA GAGGTGTGTT 22401 GCTCGCCTGC GCCATAGTCA ATACGGCCGG TCGCGAGACT GGGGGCGTAC CGAGCGGACG CGGTATCAGT TATGCCGGCC AGCGCTCTGA CCCCCGCATG 22451 ACTGGATGGC CTTTGCCTGG AACCCGCACT CAAAAACATG CTACCTCTTT TGACCTACCG GAAACGGACC TTGGGCGTGA GTTTTTGTAC GATGGAGAAA 22501 GAGCCCTTTG GCTTTTCTGA CCAGCGACTC AAGCAGGTTT ACCAGTTTGA CTCGGGAAAC CGAAAAGACT GGTCGCTGAG TTCGTCCAAA TGGTCAAACT 22551 GTACGAGTCA CTCCTGCGCC GTAGCGCCAT TGCTTCTTCC CCCGACCGCT CATGCTCAGT GAGGACGCGG CATCGCGGTA ACGAAGAAGG GGGCTGGCGA 22601 GTATAACGCT GGAAAAGTCC ACCCAAAGCG TACAGGGGCC CAACTCGGCC CATATTGCGA CCTTTTCAGG TGGGTTTCGC ATGTCCCCGG GTTGAGCCGG 22651 GCCTGTGGAC TATTCTGCTG CATGTTTCTC CACGCCTTTG CCAACTGGCC CGGACACCTG ATAAGACGAC GTACAAAGAG GTGCGGAAAC GGTTGACCGG 22701 CCAAACTCCC ATGGATCACA ACCCCACCAT GAACCTTATT ACCGGGGTAC GGTTTGAGGG TACCTAGTGT TGGGGTGGTA CTTGGAATAA TGGCCCCATG

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Figure 26 X

22801 CAGGA GC TCTACAGCTT CCTGGAGCGC CACTUGUCUI INC GTCCTTGTCG AGATGTCGAA GGACCTCGCG GTGAGCGGGA TGAAG 22851 CCACAGTGCG CAGATTAGGA GCGCCACTTC TTTTTGTCAC TTGAA GGTGTCACGC GTCTAATCCT CGCGGTGAAG AAAAACAGTG AACTT	AAACA
22901 TGTAAAATA ATGTACTAGA GACACTTTCA ATAAAGGCAA ATGCT ACATTTTAT TACATGATCT CTGTGAAAGT TATTTCCGTT TACGA	
22951 TTGTACACTC TCGGGTGATT ATTTACCCCC ACCCTTGCCG TCTGC AACATGTGAG AGCCCACTAA TAAATGGGGG TGGGAACGGC AGACG	CGGCA
23001 TTAAAAATCA AAGGGGTTCT GCCGCGCATC GCTATGCGCC ACTGG AATTTTTAGT TTCCCCAAGA CGGCGCGTAG CGATACGCGG TGACC	CAGGG GTCCC
23051 ACACGTTGCG ATACTGGTGT TTAGTGCTCC ACTTAAACTC AGGCA TGTGCAACGC TATGACCACA AATCACGAGG TGAATTTGAG TCCGT	
23101 ATCCGCGGCA GCTCGGTGAA GTTTTCACTC CACAGGCTGC GCACC TAGGCGCCGT CGAGCCACTT CAAAAGTGAG GTGTCCGACG CGTGG	CATCAC STAGTG
23151 CAACGCGTTT AGCAGGTCGG GCGCCGATAT CTTGAAGTCG CAGTT	CCCCG
23201 CTCCGCCCTG CGCGCGCGAG TTGCGATACA CAGGGTTGCA GCACT GAGGCGGGAC GCGCGCGCTC AACGCTATGT GTCCCAACGT CGTGA	rggaac Accttg
23251 ACTATCAGCG CCGGGTGGTG CACGCTGGCC AGCACGCTCT TGTCC TGATAGTCGC GGCCCACCAC GTGCGACCGG TCGTGCGAGA ACAGC	GAGAT
23301 CAGATCCGCG TCCAGGTCCT CCGCGTTGCT CAGGGCGAAC GGAGT	CAACT
GTCTAGGCGC AGGTCCAGGA GGCGCAACGA GTCCCGCTTG CCTCA 23351 TTGGTAGCTG CCTTCCCAAA AAGGGCGCGT GCCCAGGCTT TGAGT	TTGCAC
AACCATCGAC GGAAGGGTTT TTCCCGCGCA CGGGTCCGAA ACTCA	
AGCGTGGCAT CACCGTAGTT TTCCACTGGC ACGGGCCAGA CCCGG 23451 ATACAGCGCC TGCATAAAAG CCTTGATCTG CTTAAAAGCC ACCTG	CAATCC
TATGTCGCGG ACGTATTTTC GGAACTAGAC GAATTTTCGG TGGA	CTCGGA
23501 TTGCGCCTTC AGAGAAGAAC ATGCCGCAAG ACTTGCCGGA AAAC AACGCGGAAG TCTCTTCTTG TACGGCGTTC TGAACGGCCT TTTG	ACTAAC
23551 GCCGGACAGG CCGCGTCGTG CACGCAGCAC CTTGCGTCGG TGTTC CGGCCTGTCC GGCGCAGCAC GTGCGTCGTG GAACGCAGCC ACAA	GGAGAT CCTCTA
23601 CTGCACCACA TTTCGGCCCC ACCGGTTCTT CACGATCTTG GCCT GACGTGGTGT AAAGCCGGGG TGGCCAAGAA GTGCTAGAAC CGGA	TGCTAG ACGATC
23651 ACTGCTCCTT CAGCGCGCGC TGCCCGTTTT CGCTCGTCAC ATCC TGACGAGGAA GTCGCGCGCG ACGGGCAAAA GCGAGCAGTG TAGG	ATTTCA TAAAGT

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23701	AGTGCT	CCTTATTTAT	CATAATCCTT	CCCTCT	ACTTAAGCTC
23701					
	TAGTGCACGA	GGAATAAATA	GTATTACGAA	GGCACATCTG	TGAATTCGAG
23751	GCCTTCGATC	TCAGCGCAGC	GGTGCAGCCA	CAACGCGCAG	CCCGTGGGCT
35.55	= :			GTTGCGCGTC	
23801	CGTGATGCTT	GTAGGTCACC	TCTGCAAACG	ACTGCAGGTA	CGCCTGCAGG
	GCACTACGAA	CATCCAGTGG	AGACGTTTGC	TGACGTCCAT	GCGGACGTCC
. 23851	AATCGCCCCA	TCATCGTCAC	AAAGGTCTTG	TTGCTGGTGA	AGGTCAGCTG
	TTAGCGGGGT	AGTAGCAGTG	TTTCCAGAAC	AACGACCACT	TCCAGTCGAC
23901	CAACCCGCGG	TGCTCCTCGT	TCAGCCAGGT	CTTGCATACG	GCCGCCAGAG
33772				GAACGTATGC	
23951				TCGCCTTTAG	
	GAAGGTGAAC	CAGTCCGTCA	TCAAACTTCA	AGCGGAAATC	TAGCAATAGG
24001	ACGTGGTACT	TGTCCATCAG	CGCGCGCGCA	GCCTCCATGC	CCTTCTCCCA
	TGCACCATGA	ACAGGTAGTC	GCGCGCGCGT	CGGAGGTACG	GGAAGAGGGT
24051	CCCAGACACG	ATCCCCACAC	TCAGCGGGTT	CATCACCGTA	ልጥጥጥር ል ርጥጥጥ
24031				GTAGTGGCAT	
	3631313131				
24101	CCGCTTCGCT	GGGCTCTTCC	TCTTCCTCTT	GCGTCCGCAT	ACCACGCGCC
	GGCGAAGCGA	CCCGAGAAGG	AGAAGGAGAA	CGCAGGCGTA	TGGTGCGCGG
24151	ACTGGGTCGT	CTTCATTCAG	CCGCCGCACT	GTGCGCTTAC	CTCCTTTGCC
	TGACCCAGCA	GAAGTAAGTC	GGCGGCGTGA	CACGCGAATG	GAGGAAACGG
24201	ATGCTTGATT	AGCACCGGTG	GGTTGCTGAA	ACCCACCATT	TGTAGCGCCA
				TGGGTGGTAA	
24251				TTACCTCTGG	
	GTAGAAGAGA	AAGAAGGAGC	GACAGGTGCT	AATGGAGACC	ACTACCGCCC
24301	CGCTCGGGCT	TGGGAGAAGG	GCGCTTCTTT	TTCTTCTTGG	GCGCAATGGC
	GCGAGCCCGA	ACCCTCTTCC	CGCGAAGAAA	AAGAAGAACC	CGCGTTACCG
24353	amaaaaa	0000100000	**************************************	COMCCCMCMC	0000000000
24351				CGACCCACAC	CGCGGCACCA
	0111A00C00	COSCICCAGE	270000000		0000001001
24401	GCGCGTCTTG	TGATGAGTCT	TCCTCGTCCT	CGGACTCGAT	ACGCCGCCTC
					TGCGGCGGAG
24451	አጥርርርርጥጥጥጥ	איזיכיניניניניני	ררפינים אפניר	GCCGCCGACG	GGGACGGGGA
24471				CCGCCGCTGC	
	TAGGCGAAAA	AACCCCCGCG	GGCCCCTCCG	CCGCCGC1GC	CCC1GCCC1
24501	CGACACGTCC	TCCATGGTTG	GGGGACGTCG	CGCCGCACCG	CGTCCGCGCT
	GCTGTGCAGG	AGGTACCAAC	CCCCTGCAGC	GCGGCGTGGC	GCAGGCGCGA
24551	CCCCCCTCCT	ም ተር ርርር ርርር ርርርር ርርርር ርርርርር ርርርርርርርርርርር	ጥርርጥርጥጥርር	GACTGGCCAT	ተ ጥር ር ጥጥር ጥር ር
2433T					AAGGAAGAGG
	GULLUCALUA	ANGUGUALG	DUUMAUAUU	CIGACCOGIA	
24601	TATAGGCAGA	AAAAGATCAT	GGAGTCAGTC	GAGAAGAAGG	ACAGCCTAAC
24001					TGTCGGATTG

Figure 262

24701		-	GCACCCCGC CGTGGGGGCG		
24751			TGTAAGCGAA		
	TAGCTCGTCC	TGGGTCCAAA	ACATTCGCTT	CIGCIGCICC	TGGCGAGTCA
24801			AAGACCAGGA TTCTGGTCCT		
24851			GAAAGGCATG CTTTCCGTAC		
24901	••••		TCTGCAGCGC AGACGTCGCG		
24951			ATGTGCCCCT TACACGGGGA		
25001			TTCTCACCGC AAGAGTGGCG		
25051			CAACCCGCGC GTTGGGCGCG		
25101			CCACCTATCA GGTGGATAGT		
25151			GCCAACCGCA CGGTTGGCGT		
25201			CATACCTGAT GTATGGACTA		
25251	•		TTGGACGCGA AACCTGCGCT		
25301		• • • • • • • • • • • • • • • • • • • •	GAAAATGAAA CTTTTACTTT		
25351	GAACTCGAGG CTTGAGCTCC		GCGCCTAGCC CGCGGATCGG		
25401	GGTCACCCAC CCAGTGGGTG		CGGCACTTAA GCCGTGAATT		
25,451	GCACAGTCAT CGTGTCAGTA		ATCGTGCGCC TAGCACGCGG		
25501	GATGCAAATT CTACGTTTAA		AACAGAGGAG TTGTCTCCTC		
25551	CGAGCAGCTA GCTCGTCGAT				GACTTGGAGG CTGAACCTCC

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25651		-		ATGCAGCGCA TACGTCGCGT	
25701		• • • • • • • • •		CGTACGCCAG GCATGCGGTC	
05751				CCTACCTTGG	
25751				GGATGGAACC	
25801				TCCACGCTCA AGGTGCGAGT	
25851				CTTATTTCTA GAATAAAGAT	
25901				GCTTGGAGGA CGAACCTCCT	
25951	AAGGAGCTGC TTCCTCGACG			TTGAAGGACC AACTTCCTGG	
26001				GGCGGACATC CCGCCTGTAG	•
26051				TGCCAGACTT ACGGTCTGAA	
26101				CTAGAGCGCT GATCTCGCGA	
26151				CTTTGTGCCC GAAACACGGG	
26201				GCTACCTTCT CGATGGAAGA	
26251				GAAGACGTGA CTTCTGCACT	
		ACAGTGACAG	CGACGTTGGA	TACGTGGGGC	GTGGCGAGGG
		AAGCGTCGAC	GAATTGCTTT	CAGTTTAATA	GCCATGGAAA
26401	GAGCTGCAGG CTCGACGTCC			TCCGCGGCTC AGGCGCCGAG	
		CCCGACACCT	GCAGCCGAAT	GGAAGCGTTT	AAACATGGAC
26501				ACGAAGACCA TGCTTCTGGT	ATCCCGCCCG TAGGGCGGGC

Figure 26 AB

WO 02/022080					PCT/US01/28861
26551	co	AGCTTACCGC	СТСССТСАТТ	ACCCAGG	
20301		TCGAATGGCG			
26601	CCAATTGCAA	GCCATCAACA	AAGCCCGCCA	AGAGTTTCTG	CTACGAAAGG
	GGTTAACGTT	CGGTAGTTGT	TTCGGGCGGT	TCTCAAAGAC	GATGCTTTCC
26651	GACGGGGGGT	TTACTTGGAC	CCCCAGTCCG	GCGAGGAGCT	CAACCCAATC
		AATGAACCTG			
26701	CCCCGCCGC				
	GGGGGCGCG	GCGTCGGGAT	AGTCGTCGTC	GGCGCCCGGG	AACGAAGGGT
26751	GGATGGCACC	CAAAAAGAAG	CTGCAGCTGC	CGCCGCCACC	CACGGACGAG
	CCTACCGTGG	GTTTTTCTTC	GACGTCGACG	GCGGCGGTGG	GTGCCTGCTC
26801	GAGGAATACT				
		CCCTGTCAGT			
26851	GGACATGATG				
26001	AAGAGGTGTC	CTTCTGACCC			
26901		TCTGCTTTGT			
	TICICCACAG	1010011101		000110001121	0000.100000
26951	GCGCCCCAGA	AATCGGCAAC	CGGTTCCAGC	ATGGCTACAA	CCTCCGCTCC
		TTAGCCGTTG			
27001		CCGGCACTGC			
		GGCCGTGACG			
27051	CCACTGGAAC				
07101		GTCCCGGCCA			
27101	GAGCAACAAC	TCGCGGTTCC			
27151			•		
2/151	CATAGTTGCT	ACGAACGTTC			
27201	GCTTTCTTCT				
		GATGGTAGTG			
· 27251	TACTACCGTC				
		TAGAGATGTC			
27301	CAGCAGCGGC				
	GTCGTCGCCG	GTGTGTCTTC	GTTTCCGCTG	GCCTATCGTT	CTGAGACTGT
27351	AAGCCCAAGA				
		TTAGGTGTCG			
27401	TCTGGCGCCC				
					TTGTCCTAAA
27451	TTCCCACTCT				
	AAGGGTGAGA	CATACGATAT	AAAGTTGTCT	CGTCCCCGGT	TCTTGTTCTC

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27551	TCACAAAAGC	GAAGATCAGC	TTCGGCGCAC	GCTGGAAGAC	GCGGAGGCTC
2.30-		CTTCTAGTCG			
	AGIGITICG	CIICIMOICO	71.00000010		00001000
00601	mommo: 0m; ;	ATACTGCGCG		3.CC3.CT3.CTT	TO COCOCOTO
27601					
	AGAAGTCATT	TATGACGCGC	GACTGAGAAT	TCCTGATCAA	AGCGCGGAA
27651		AAGCGCGAAA			
	AGAGTTTAAA	TTCGCGCTTT	TGATGCAGTA	GAGGTCGCCG	GTGTGGGCCG
27701	GCCAGCACCT	GTTGTCAGCG	CCATTATGAG	CAAGGAAATT	CCCACGCCCT
2		CAACAGTCGC			
		5.2.55	•••••	•	
27751	N C N T C T C C N C	TTACCAGCCA	СУУУТСССУС	TTCCCCCTCC	AGCTGCCCAA
27731		AATGGTCGGT			
	TGTACACCTC	AATGGTCGGT	GITIACCCIG	MCGCCGMCC	1CGACGGG11
			CM) C) MC) CC	CCCCCACCCC	እ <i>ር እ ጥር እ</i> ጥልጥር
27801		CCCGAATAAA			
	CTGATGAGTT	GGGCTTATTT	GATGTACTCG	CGCCCTGGGG	TGTACTATAG
27851		GGAATACGCG			
	GGCCCAGTTG	CCTTATGCGC	GGGTGGCTTT	GGCTTAAGAG	GACCTTGTCC
27901		CACCACACCT			
	GCCGATAATG	GTGGTGTGGA	GCATTATTGG	AATTAGGGGC	ATCAACCGGG
27951	GCTGCCCTGG	TGTACCAGGA	AAGTCCCGCT	CCCACCACTG	TGGTACTTCC
		ACATGGTCCT			
28001	CAGAGACGCC	CAGGCCGAAG	TTCAGATGAC	TAACTCAGGG	GCGCAGCTTG
20001	CTCTCTCCCC	GTCCGGCTTC	AAGTCTACTG	ATTGAGTCCC	CGCGTCGAAC
	6101010000	9100000110		***************************************	
28051	CCCCCCCC	TCGTCACAGG	CTCCCCTCCC	CCGGGCAGGG	ТАТААСТСАС
28031		AGCAGTGTCC			
	GCCCGCCGAA	MGCMG1G1CC	CACGCCAGCG	6666667666	
		GAGGGCGAGG	mammea ceme	33CC3CC3CT	CCCTC A CCTC
28101					
	GACTGTTAGT	CTCCCGCTCC	ATAAGTCGAG	TIGUTGUTCA	GCCAC ICGAG
	•				000000000
28151	CTCGCTTGGT	CTCCGTCCGG	ACGGGACATT	TCAGATCGGC	GGCGCCGGCC
	GAGCGAACCA	GAGGCAGGCC	TGCCCTGTAA	AGTCTAGCCG	CCGCGGCCGG
28201	GCTCTTCATT	CACGCCTCGT	CAGGCAATCC	TAACTCTGCA	GACCTCGTCC
	CGAGAAGTAA	GTGCGGAGCA	GTCCGTTAGG	ATTGAGACGT	CTGGAGCAGG
28251	TCTGAGCCGC	GCTCTGGAGG	CATTGGAACT	CTGCAATTTA	TTGAGGAGTT
	AGACTCGGCG	CGAGACCTCC	GTAACCTTGA	GACGTTAAAT	AACTCCTCAA
28301	ጥርጥር ርር ልጥርር	GTCTACTTTA	ACCCCTTCTC	GGGACCTCCC	GGCCACTATC
20301					CCGGTGATAG
	MCMCGG1 MGC	CUCUIGUUUI	. OGGGRANONG		
00055	000000000000000000000000000000000000000		TATATACE & COCOCO	. TA A A CC A CTC	GGCGGACGGC
28351	CGGATCAATT	INTICIANC	777000000		CCGCCTGCCG
	GCCTAGTTAA	ATAAGGATTG	AAAC TGCGCC	ATTICCIONG	
				- C. S. OMCCOCC	MC3 3 3 C 3 C C C C
28401					TGAAACACCT
	ATGCTGACTT	, YCYVLLCYCC	TCTCCGTCTC	GTTGACGCGG	ACTTTGTGGA

Figure 26 AD 84/144

WO 02/022080					PCT/US01/28861
28451				CCGCGACT	
28501	GCTACTITGA	ATTGCCCGAG	GATCATATCG	AGGCCCGGC	GCACGGCGTC
				TCCCGGGCCG	
28551				AGCCTGATTC	
				TCGGACTAAG	
28601				GGGACCCTGT	
				CCCTGGGACA	
28651				ATCAAGATCT	
				TAGTTCTAGA	
28701				TAAAATATAC	
				ATTTTATATG	
28751				CCCGCCCAAG GGGCGGGTTC	
				CCCTCTGTGA	
28801				GGGAGACACT	
28851				GAACCTCTCC	
				CTTGGAGAGG	
28901				CCTGCCGGGA	
			4	GGACGGCCCT	
28951				CCTGACCGTA	
				GGACTGGCAT	
29001				CCAGAACAGG	
				GGTCTTGTCC	
29051				GCAGCTACTG	
				CGTCGATGAC	
29101				TAATTCAGGT	
				ATTAAGTCCA	
29151				TTCTCTTTAT	
				AAGAGAAATA	
29201	ACGCTTCTCT	GCCTAAGGCT	CGCCGCCTGC	TGTGTGCACA	TTTGCATTTA
				ACACACGTGT	
29251	TTGTCAGCTT	TTTAAACGCT	GGGGTCGCCA	CCCAAGATGA	TTAGGTACAT
					AATCCATGTA
29301					ACCCAAAAGG
				GGTGCCATGG	
29351					TGAAGCTAAT
	ACCTAAAATT	CCTCGGTCGG	ACATTACAAT	GTAAGCGTCG	ACTTCGATTA

29451	TCGCCACAAA	AACAAAATTG	GCAAGTATGC	TGTTTATGCT	ATTTGGCAGC
	ACCCCTGTTT	TTGTTTTAAC	CGTTCATACG	ACAAATACGA	TAAACCGTCG
	AGCGG10111	11011111110	COLICAINCO	ACABITACOA	
00501	a	m> C> C> Cm> m	11momm1010	mmmmca> Ccc	M111100010
29501		TACAGAGTAT			
	GTCCACTGTG	ATGTCTCATA	TTACAATGTC	AAAAGGTCCC	ATTTTCAGTA
				•	
29551	AAAACTTTTA	TGTATACTTT	TCCATTTTAT	GAAATGTGCG	ACATTACCAT
	TTTTGAAAAT	ACATATGAAA	AGGTAAAATA	CTTTACACGC	TGTAATGGTA
20601	CM3 C3 MC3 CC	AAACAGTATA	* COMCOCCC	CCCACAAAAT	か にかとかといる ある
29601	-				
	CATGTACTCG	TTTGTCATAT	TCAACACCGG	GGGTGTTTTA	ACACACCTTT
29651	ACACTGGCAC	TTTCTGCTGC	ACTGCTATGC	TAATTACAGT	GCTCGCTTTG
	TGTGACCGTG	AAAGACGACG	TGACGATACG	ATTAATGTCA	CGAGCGAAAC
29701	GTCTGTACCC	TACTCTATAT	TAAATACAAA	AGCAGACGCA	GCTTTATTGA
	CAGACATGGG	ATGAGATATA	ATTTATGTTT	TCGTCTGCGT	CGAAATAACT
	0				
29751	CC3333C333	ATGCCTTAAT	<u>ጥጥል</u> ርጥል ልርጥጥ	ACAAACCTAA	тстелеслет
29/31	••	TACGGAATTA			
	CCTTTTCTTT	TACGGAATTA	MATGATICAM	IGITICGATI	ACAGIGGIGA
29801	AACTGCTTTA	CTCGCTGCTT	GCAAAACAAA	TTCAAAAAGT	TAGCATTATA
	TTGACGAAAT	GAGCGACGAA	CGTTTTGTTT	AAGTTTTTCA	ATCGTAATAT
					•
29851	ATTAGAATAG	GATTTAAACC	CCCCGGTCAT	TTCCTGCTCA	ATACCATTCC
	TAATCTTATC	CTAAATTTGG	GGGGCCAGTA	AAGGACGAGT	TATGGTAAGG
		T 112 T 111 11 11 11 11 11 11 11 11 11 11 11		•	
29901	ССТСААСААТ	TGACTCTATG	тесертатес	ጥርርልርርርርጥል	СААССТТСАА
23302	• •	ACTGAGATAC			
	GGACIIGIIA	ACIGAGAIAC	ACCCIAIACG	AGGICGCGAI	GIIGGAACII
29951		CTGGATGTCA			
	CAGTCCGAAG	GACCTACAGT	CGTAGACTGA	AACCGGTCGT	GGACAGGGCG
30001	GGATTTGTTC	CAGTCCAACT	ACAGCGACCC	ACCCTAACAG	AGATGACCAA
	CCTAAACAAG	GTCAGGTTGA	TGTCGCTGGG	TGGGATTGTC	TCTACTGGTT
	•		•		
30051	CACHACCAAC	GCGGCCGCCG	CTACCGGACT	TACATCTACC	ACAAATACAC
30031		CGCCGGCGGC			
	0101190119	COCCOGCOGC	GNIGGCCIGN	AIGIAGAIGG	10111111010
20101	CCCAAGTTTC	maca a membana	*****	> M > > CMMCCC	CARCINCETTCC
30101					
	GGGTTCAAAG	ACGGAAACAG	TTATTGACCC	TATTGAACCC	GTACACCACC
30151	TTCTCCATAG				
	AAGAGGTATC	GCGAATACAA	ACATACGGAA	TAATAATACA	CCGAGTAGAC
				•	
30201	CTGCCTAAAG	CGCAAACGCG	CCCGACCACC	CATCTATAGT	CCCATCATTG
		GCGTTTGCGC			
	GUCGGUIIIC	20222600		J	
20051	maams as a a	***	CCXXMCC3M3	CAMMOCACOC	ACTONANCAC
30251	TGCTACACCC				
	ACGATGTGGG	TTTGTTACTA	CCTTAGGTAT	CTAACCTGCC	TGACTTTGTG
30301	ATGTTCTTTT				
	TACAAGAAAA	GAGAATGTCA	TACTAATTTA	CTCTGTACTA	AGGAGCTCAA

Figure 26 AF

30401	TGCGGTTTCT	CACATCGAAG	TAGACTGCAT	TCCAGCCTTC	ACAGTCTATT
50102		GTGTAGCTTC			
	ACGCCAAAGA	GIGIAGETIC	AICIGACOIA	70016001110	101010111121
				mamaaa aaam	0.00.0000
30451		ATTTGTCACC			
	ACGAAATGCC	TAAACAGTGG	GAGTGCGAGT	AGACGTCGGA	GTAGTGACAC
30501	GTCATCGCCT	TTATCCAGTG	CATTGACTGG	GTCTGTGTGC	GCTTTGCATA
	CAGTAGCGGA	AATAGGTCAC	GTAACTGACC	CAGACACACG	CGAAACGTAT
30551	TOTOLOGICACIO	CATCCCCAGT	ACAGGGACAG	СУСТАТАССТ	CACCTTCTTA
.50551		GTAGGGGTCA			
	AGAGICIGIG	GIAGGGGICA	1616661616	CIGNINICGN	CICOMONI
				*****************************	> mm > mmmcc >
30601		ATTATGAAAT			
	CTTAAGAAAT	TAATACTTTA	AATGACACTG	AAAAGACGAC	TAATAAACGT
30651		GTTTTGTTCC			
	GGGATAGACG	CAAAACAAGG	GGCTGGAGGT	TCGGAGTTTC	TGTATATAGT
30701	TGCAGATTCA	CTCGTATATG	GAATATTCCA	AGTTGCTACA	ATGAAAAAAG
	ACGTCTAAGT	GAGCATATAC	CTTATAAGGT	TCAACGATGT	TACTTTTTTC
30751	ССВТСТТТСС	GAAGCCTGGT	TATATGCAAT	CATCTCTGTT	ATGGTGTTCT
30731		CTTCGGACCA			
	GCIAGAAAGG	CIICOGACCA	Ainincoitin	010	11.001.012.01
20001	0010010010	CTTAGCCCTA	CCMAMAMAMC	CCTACCTTCA	CATTCCCTCC
30801		GAATCGGGAT			
٠.	CGTCATGGTA	GAATCGGGAT	CGATATATAG	GGATGGAACT.	GIWACCGACC
30851		ATGCCATGAA			
	TTGCGTTATC	TACGGTACTT	GGTGGGTTGA	AAGGGGCGCG	GGCGATACGA
					•
30901		CAAGTTGTTG			
	AGGTGACGTT	GTTCAACAAC	GGCCGCCGAA	ACAGGGTCGG	TTAGTCGGAG
30951	GCCCACCTTC	TCCCACCCC	ACTGAAATCA	GCTACTTTAA	TCTAACAGGA
		AGGGTGGGG			
	2000100.210				
31001	CCACATCACT	GACACCCTAG	ארכידאכאאאי	GGACGGAATT	ATTACAGAGO
31001		CTGTGGGATC			
	CCICIACIGA	CIGIGGAIC	INGAICIIIA	ccigcciins	THE TOTAL CO
24254			100001000	CCCACCAACA	CCCCAMCAAT
31051		AGAAAGACGC			
	TCGCGGACGA	TCTTTCTGCG	TCCCGTCGCC	GGCTCGTTGT	CGCGTACTTA
31101		AAGACATGGT			
	GTTCTCGAGG	TTCTGTACCA	ATTGAACGTG	GTCACGTTTT	CCCCATAGAA
		AAGCAGGCCA			
		TTCGTCCGGT			
		 _			
31201	ACCGCCTTAG	CTACAAGTTG	CCAACCAAGC	GTCAGAAATT	GGTGGTCATG
		GATGTTCAAC			
	1 GOCOGNATC	oni di i cinto			
21251	C#CCC3 C3 3 3	AGCCCATTAC	СУФУУСТСУС	ር <u>እ</u> ርጥርርርጥ <u>እ</u> ር	AAACCGAAGG
21721		TCGGGTAATG			
	CACCCTCTTT	TUGGGTAATG	GIMITGAGTC	GIGAGCCAIC	1110001100

Figure 24 A6 87/144

31351	AGACCCTGTG	CGGTCTCAAA	GATCTTATTC	ССТТТААСТА	ATAAAAAAAA
					TATTTTTTTT
	1 C 1 GGGACAC	accionatii	CINONNIANO	GGAAATIGAT	IMITITITI
21401					
31401					CTGTCCAGTT
	TATTATTTCG	TAGTGAATGA	ATTTTAGTCA	ATCGTTTAAA	GACAGGTCAA
31451	TATTCAGCAG	CACCTCCTTG	CCCTCCTCCC	AGCTCTGGTA	TTGCAGCTTC
	ATAAGTCGTC	GTGGAGGAAC	GGGAGGAGGG	TCGAGACCAT	AACGTCGAAG
					•
31501	СТССТСССТС	САААСТОТСТ	CCACAATCTA	AATGGAATGT	CACTTTCCTC
01301			GGTGTTAGAT	• • •	
	GAGGACCGAC	GIIIGAAAGA	GGIGIIAGAI	TIACCTIACA	GICAMAGGAG
31551		CONTROCCONO	CCACTATCTT	0.mammamma	a.a.aaaa
31331					
	GACAAGGACA	GGTAGGCGTG	GGTGATAGAA	GTACAACAAC	GTCTACTTCG
31601			ACCTTCAACC		
	CGCGTTCTGG	CAGACTTCTA	TGGAAGTTGG	GGCACATAGG	TATACTGTGC
31651	GAAACCGGTC	CTCCAACTGT	GCCTTTTCTT	ACTCCTCCCT	TTGTATCCCC
	CTTTGGCCAG	GAGGTTGACA	CGGAAAAGAA	TGAGGAGGGA	AACATAGGGG
31701	CAATGGGTTT	CAAGAGAGTC	CCCCTGGGGT	ACTCTCTTTG	CGCCTATCCG
•			GGGGACCCCA		
31751	AACCTCTAGT	TACCTCCAAT	GGCATGCTTG	СССТСАВВЕТ	GGGCAACGGC
01.01			CCGTACGAAC		
	IIGONGAICA	A1GOAGG11A	CCGIACGAAC	GCGAGIIIIA	cccorracco
31801		A CCA CCCCCC	CAACCTTACC	TCCCA A A A TC	ma a coa coco
31801					
	GAGAGAGACC	TGCTCCGGCC	GTTGGAATGG	AGGGTTTTAC	ATTGGTGACA
31851			CCAAGTCAAA		
	CTCGGGTGGA	GAGTTTTTTT	GGTTCAGTTT	GTATTTGGAC	CTTTATAGAC
31901			GAAGCCCTAA		
	GTGGGGAGTG	TCAATGGAGT	CTTCGGGATT	GACACCGACG	GCGGCGTGGA
31951	CTAATGGTCG	CGGGCAACAC	ACTCACCATG	CAATCACAGG	CCCCGCTAAC
	GATTACCAGC	GCCCGTTGTG	TGAGTGGTAC	GTTAGTGTCC	GGGGCGATTG
•					
32001	CGTGCACGAC	TCCAAACTTA	GCATTGCCAC	CCAAGGACCC	CTCACAGTGT
			CGTAACGGTG		
	OCACG1GC1G	7001110/241	COLMCGGIG	0011001000	ONG 101 CACA
32051	CAGAAGGAAA	CCTACCCCTC	CAAACAMCAC	CCCCCTCAC	CACCACCCAM
32031					
	GTCTTCCTTT	CGATCGGGAC	GTTTGTAGTC	CGGGGGAGTG	GTGGTGGCTA
••••					
32101	AGCAGTACCC				
	TCGTCATGGG	AATGATAGTG	ACGGAGTGGG	GGAGATTGAT	GACGGTGACC
32151	TAGCTTGGGC				
	ATCGAACCCG	TAACTGAACT	TTCTCGGGTA	AATATGTGTT	TTACCTTTTG
32201	TAGGACTAAA	GTACGGGGCT	CCTTTGCATG	TAACAGACGA	CCTAAACACT
	ATCCTGATTT	CATGCCCCGA	GGAAACGTAC	ATTGTCTGCT	GGATTTGTGA

Figure 26 AH

32301	AACTAAAGTT	ACTGGAGCCT	TGGGTTTTGA	TTCACAAGGC	AATATGCAAC
32302		TGACCTCGGA			
	TIGNITICAM	IGACCICGGA	ACCCAAAACI	ANGIGITECG	ITATACGITG
32351		AGGAGGACTA			
	AATTACATCG	TCCTCCTGAT	TCCTAACTAA	GAGTTTTGTC	TGCGGAATAT
32401	CTTC ATCTTA	GTTATCCGTT	TCATCCTCAA	AACCAACTAA	ATCTAACACT
32401	••••	CAATAGGCAA			
	GAACTACAAT	CAATAGGCAA	ACTACGAGTT	TIGGIIGAII	IAGATICIGA
32451	AGGACAGGGC	CCTCTTTTTA	TAAACTCAGC	CCACAACTTG	GATATTAACT
	TCCTGTCCCG	GGAGAAAAT	ATTTGAGTCG	GGTGTTGAAC	CTATAATTGA
		•••••			
		CCTTTACTTG	mmm > C > C C mm	CAAACAAMMC	C
32501					
	TGTTGTTTCC	GGAAATGAAC	AAATGTCGAA	GTTTGTTAAG	GTTTTTCGAA
32551	GAGGTTAACC	TAAGCACTGC	CAAGGGGTTG	ATGTTTGACG	CTACAGCCAT
	CTCCAATTGG	ATTCGTGACG	GTTCCCCAAC	TACAAACTGC	GATGTCGGTA
	0.00.2				
32601	NCCC NMMN NM	GCAGGAGATG	CCCTTCAATT	TECTTCACCT	AATGCACCAA
32601					
	TCGGTAATTA	CGTCCTCTAC	CCGAACTTAA	ACCAAGIGGA	TIACGIGGII
32651		CCTCAAAACA			
	TGTGTTTAGG	GGAGTTTTGT	TTTTAACCGG	TACCGGATCT	TAAACTAAGT
				•	
32701	AACAACCCTA	TGGTTCCTAA	ACTAGGAACT	GGCCTTAGTT	TTGACAGCAC
32/01		ACCAAGGATT			
	TTGTTCCGAT	ACCAAGGATT	IGAICCIIGA	CCGGAAICAA	MC101C010
32751					ACTTTGTGGA
	TCCACGGTAA	TGTCATCCTT	TGTTTTTATT	ACTATTCGAT	TGAAACACCT
32801	CCACACCAGC	TCCATCTCCT	AACTGTAGAC	TAAATGCAGA	GAAAGATGCT
32001					CTTTCTACGA
	GGIGIGGICG	AGGIAGAA	IIGACAICIG	Allincole:	C111C1CO
32851					TTGCTACAGT
	TTTGAGTGAA	ACCAGAATTG	TTTTACACCG	TCAGTTTATG	AACGATGTCA
32901	TTCAGTTTTG	GCTGTTAAAG	GCAGTTTGGC	TCCAATATCT	GGAACAGTTC
02302					CCTTGTCAAG
	MAGICAMAC	CONCHILLIC	C01C.22.000	110011111111	
			> C > MMMC > C C	3.3.3.MCC3.CT	CCMACMAAAC
32951					GCTACTAAAC
	TTTCACGAGT	AGAATAATAT	TCTAAACTGC	TTTTACCTCA	CGATGATTTG
33001	AATTCCTTCC	TGGACCCAGA	ATATTGGAAC	TTTAGAAATG	GAGATCTTAC
	TTAAGGAAGG	ACCTGGGTCT	TATAACCTTG	AAATCTTTAC	CTCTAGAATG
	1111100:2:00				
22654	0011000101	CCCM3/M3/C3/3	A C C C T C T T T C C	አጥጥጥ አጥር ር ር ጥ	AACCTATCAG
33051	TGAAGGCACA	GCCTATACAA	WCGC 1G11GG	MILIMIUCCI	WWCCTWICWG
	ACTTCCGTGT	CGGATATGTT	TGCGACAACC	TAAATAUGGA	TTGGATAGTC
33101	CTTATCCAAA	ATCTCACGGT	AAAACTGCCA	AAAGTAACAT	TGTCAGTCAA
	GAATAGGTTT	TAGAGTGCCA	TTTTGACGGT	TTTCATTGTA	ACAGTCAGTT
	J				
22253	COMMA COMA	3000303033	33CT333CCT	CTABCSCTAS	CCATTACACT
33151					
	CAAATGAATT	TGCCTCTGTT	TTGATTTGGA	CATTGTGATT	GGTAATGTGA

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33251	CATTTTCATG	GGACTGGTCT	GGCCACAACT	ACATTAATGA	AATATTTGCC
33232		CCTGACCAGA			
	GINAMAGIAC	CCIGACCAGA	ccddidiida		111111111111111111111111111111111111111
				aa	C1.1 mccmmmc
33301		ACACTTTTTC			
	TGTAGGAGAA	TGTGAAAAAG	TATGTAACGG	GTTCTTATTT	CTTAGCAAAC
33351	TGTTATGTTT	CAACGTGTTT	ATTTTTCAAT	TGCAGAAAAT	TTCAAGTCAT
	ACAATACAAA	GTTGCACAAA	TAAAAAGTTA	ACGTCTTTTA	AAGTTCAGTA
	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,				
			0001001001	CAMA COMMAN	NC NC NC NC NC C
33401		GTAGTATAGC			
	AAAAGTAAGT	CATCATATCG	GGGTGGTGGT	GTATCGAATA	TGTCTAGTGG
33451		CAAACTCACA			
	CATGGAATTA	GTTTGAGTGT	CTTGGGATCA	TAAGTTGGAC	GGTGGAGGGA
33501	CCCAACACAC	AGAGTACACA	GTCCTTTCTC	CCCGGCTGGC	CTTAAAAAGC
33301		TCTCATGTGT			GAATTTTTCG
	6661161616	TCTCATGTGT	C7.00,22.0,.0		•
		GGGTAACAGA	0.5 m.5 mmcmm.5		TO A CACCOT
33551					
	TAGTATAGTA	CCCATTGTCT	GTATAAGAAT	CCACAATATA	AGGTGTGCCA
33601		GCCAAACGCT			
	AAGGACAGCT	CGGTTTGCGA	GTAGTCACTA	TAATTATTTG	AGGGGCCCGT
33651	GCTCACTTAA	GTTCATGTCG	CTGTCCAGCT	GCTGAGCCAC	AGGCTGCTGT
33031		CAAGTACAGC			
	CONGIGNATI	CAMOIACAGE	0		
		GTTGCTTAAC	00000000033	CCACAACTCC	አርርርርሞክርክሞ
33701					
	GGTTGAACGC	CAACGAATTG	CCCGCCGCTT	CCTCTTCAGG	TGCGGAIGIA
33751		TCATAATCGT			
	CCCCCATCTC	AGTATTAGCA	CGTAGTCCTA	TCCCGCCACC	ACGACGTCGT
			•		
33801	GCGCGCGAAT	AAACTGCTGC	CĠCCGCCGCT	CCGTCCTGCA	GGAATACAAC
		TTTGACGACG			
	000000117		000000000		
22051	> mooca > 0moo	TCTCCTCAGC	CATCATTCCC	»CCCCCCCCA	CCATAACCCC
33851	ATGGCAGTGG	TCTCCTCAGC	GAIGAIICGC	MCCCCCCCCC	CCTATTCCCC
	TACCGTCACC	AGAGGAGTCG	CTACTAAGCG	166666661	CGIATICCGC
33901	CCTTGTCCTC	CGGGCACAGC	AGCGCACCCT	GATCTCACTT	AAATCAGCAC
	GGAACAGGAG	GCCCGTGTCG	TCGCGTGGGA	CTAGAGTGAA	TTTAGTCGTG
33951	AGTAACTGCA	GCACAGCACC	ACAATATTGT	TCAAAATCCC	ACAGTGCAAG
	TCATTGACGT	CGTGTCGTGG	TGTTATAACA	AGTTTTAGGG	TGTCACGTTC
	10//110//00				
24001	GCGCTGTATC	CAAACCTVAT	ככרככפפארר	ACAGAACCCA	CGTGGCCATC
34001	GCGCTGTATC	GTTTCGAGTA		MCMCMMCCCM	CCACCCCTAC
	CGCGACATAG	GTTTCGAGTA	CCGCCCCTGG	1010110001	SCACCOS ING
34051	ATACCACAAG	CGCAGGTAGA	TTAAGTGGCG	ACCCCTCATA	AACACGCTGG
	TATGGTGTTC	GCGTCCATCT	AATTCACCGC	TGGGGAGTAT	TTGTGCGACC
			•		
34101	ACATAAACAT	TACCTCTTTT	GGCATGTTGT	AATTCACCAC	CTCCCGGTAC
24701	ጥርጥል ጥጥጥርጥል	ATGGAGAAAA	CCGTACAACA	TTAAGTGGTG	GAGGGCCATG
	IGTUTIBLE				

Figure 26 AJ

34201	GCTGGCCAAA	ACCTGCCCGC	CGGCTATACA	CTGCAGGGAA	CCGGGACTGG
				GACGTCCCTT	
	COACCOGIII	1000000	00000		00000101.00
					61 501 5005
34251				AACCATGGAT	
	TTGTTACTGT	CACCTCTCGG	GTCCTGAGCA	TTGGTACCTA	GTAGTACGAG
34301	CTCATCATAT	CAATGTTGGC	ACAACACAGG	CACACGTGCA	TACACTTCCT
34301				GTGTGCACGT	
	CAGIACIAIA	GIINCANCCO	1011010100	01010CAC01	MIGIOMOGA
34351	CAGGATTACA	AGCTCCTCCC	GCGTTAGAAC	CATATCCCAG	GGAACAACCC
	GTCCTAATGT	TCGAGGAGGG	CGCAATCTTG	GTATAGGGTC	CCTTGTTGGG
34401	እመጥርርጥር እ <i>እ</i> ጥ	CACCCTAAAT	CCCACACTCC	AGGGAAGACC	TCCCACCTAA
34401					
	TAAGGACTTA	GTCGCATTTA	GGGTGTGACG	TCCCTTCTGG	AGCGTGCATT
34451				TCGGGCAGCA	
	GAGTGCAACA	CGTAACAGTT	TCACAATGTA	AGCCCGTCGT	CGCCTACTAG
34501	СТССАСТАТС	GTAGCGCGGG	ጥጥርጥር ጥር	AAAAGGAGGT	AGACGATCCC
34301				TTTTCCTCCA	
	GAGGTCATAC	CATCGCGCCC	AAAGACAGAG	TITICCICCA	1010017000
					:
34551				ATCGTGTTGG	
	ATGACATGCC	TCACGCGGCT	CTGTTGGCTC	TAGCACAACC	AGCATCACAG
				•	
34601	ATGCCAAATG	GAACGCCGGA	CGTAGTCATA	TTTCCTGAAG	CAAAACCAGG
	. TACGGTTTAC				
	. IACGGIIIAC	CIIGCGGCCI	GCAICAGIAI	ADIOONCI IC	0111100100
34651				GGTCTCGCCG	
	ACGCCCGCAC	TGTTTGTCTA	GACGCAGAGG	CCAGAGCGGC	GAATCTAGCG
34701	manaman a am	N CTTCTN CTN	TATCCACTCT	CTCAAAGCAT	CCAGGCGCCC
	TUTGIGIAGI	MOLLGINGIN			
				GAGTTTCGTA	
				GAGTTTCGTA	
	AGACACATCA	TCAACATCAT	ATAGGTGAGA		GGTCCGCGGG
34751	AGACACATCA CCTGGCTTCG	TCAACATCAT GGTTCTATGT	ATAGGTGAGA AAACTCCTTC	ATGCGCCGCT	GCCCTGATAA
	AGACACATCA CCTGGCTTCG	TCAACATCAT GGTTCTATGT	ATAGGTGAGA AAACTCCTTC		GCCCTGATAA
	AGACACATCA CCTGGCTTCG GGACCGAAGC	TCAACATCAT GGTTCTATGT CCAAGATACA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG	ATGCGCCGCT TACGCGGCGA	GCCCTGATAA CGGGACTATT
	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA	ATGCGCCGCT TACGCGGCGA GCCAACCTAC	GCCCTGATAA CGGGACTATT ACATTCGTTC
34751	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA	ATGCGCCGCT TACGCGGCGA GCCAACCTAC	GCCCTGATAA CGGGACTATT ACATTCGTTC
34751	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA	ATGCGCCGCT TACGCGGCGA	GCCCTGATAA CGGGACTATT ACATTCGTTC
34751 34801	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG	GGTCCGCGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG
34751 34801	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA	GGTCCGCGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTT
34751 34801	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG	GGTCCGCGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTT
34751 34801 34851	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA
34751 34801 34851	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA
34751 34801 34851	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT CAAAATGAAG	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG
34751 34801 34851	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG
34751 34801 34851 34901	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG TTTTTTATTC AAAAAATAAG	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA GTTTTCTAAT	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT AGGTTTTGGA	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT CAAAATGAAG GTTTTACTTC	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG TAGATAATTC
34751 34801 34851 34901	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG TTTTTTATTC AAAAAATAAG TGAACGCGCT	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA GTTTTCTAAT CCCCTCCGGT	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT AGGTTTTGGA GCCGTGGTCA	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT CAAAATGAAG GTTTTACTTC AACTCTACAG	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG TAGATAATTC CCAAAGAACA
34751 34801 34851 34901	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG TTTTTTATTC AAAAAATAAG TGAACGCGCT	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA GTTTTCTAAT CCCCTCCGGT	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT AGGTTTTGGA GCCGTGGTCA	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT CAAAATGAAG GTTTTACTTC	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG TAGATAATTC CCAAAGAACA
34751 34801 34851 34901 34951	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG TTTTTTATTC AAAAAATAAG TGAACGCGCT ACTTGCGCGA	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA GTTTTCTAAT CCCCTCCGGT GGGGAGGCCA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT AGGTTTTGGA GGCGTGGTCA CCGCACCAGT	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT CAAAATGAAG GTTTTACTTC AACTCTACAG TTGAGATGTC	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG TAGATAATTC CCAAAGAACA GGTTTCTTGT
34751 34801 34851 34901 34951	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG TTTTTTATTC AAAAAATAAG TGAACGCGCT ACTTGCGCGA	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA GTTTTCTAAT CCCCTCCGGT GGGGAGGCCA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT AGGTTTTGGA GGCGTGGTCA CCGCACCAGT	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT CAAAATGAAG GTTTTACTTC AACTCTACAG TTGAGATGTC GGCTTCCAAA	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG TAGATAATTC CCAAAGAACA GGTTTCTTGT AGGCAAACGG
34751 34801 34851 34901 34951	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG TTTTTTATTC AAAAAATAAG TGAACGCGCT ACTTGCGCGA	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA GTTTTCTAAT CCCCTCCGGT GGGGAGGCCA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT AGGTTTTGGA GGCGTGGTCA CCGCACCAGT	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT CAAAATGAAG GTTTTACTTC AACTCTACAG TTGAGATGTC	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG TAGATAATTC CCAAAGAACA GGTTTCTTGT AGGCAAACGG
34751 34801 34851 34901 34951	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG TTTTTTATTC AAAAAATAAG TGAACGCGCT ACTTGCGCGA	TCAACATCAT GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA GTTTTCTAAT CCCCTCCGGT GGGGAGGCCA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT AGGTTTTGGA GGCGTGGTCA CCGCACCAGT	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT CAAAATGAAG GTTTTACTTC AACTCTACAG TTGAGATGTC GGCTTCCAAA	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG TAGATAATTC CCAAAGAACA GGTTTCTTGT AGGCAAACGG
34751 34801 34851 34901 34951 35001	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG TTTTTTATTC AAAAAATAAG TGAACGCGCT ACTTGCGCGA GATAATGGCA CTATTACCGT	GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA GTTTTCTAAT CCCCTCCGGT GGGGAGGCCA TTTGTAAGAT AAACATTCTA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT AGGTTTTGGA GCCGTGGTCA CCGCACCAGT GTTGCACAAT CAACGTGTTA	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT CAAAATGAAG GTTTTACTTC AACTCTACAG TTGAGATGTC GGCTTCCAAA CCGAAGGTTT	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG TAGATAATTC CCAAAGAACA GGTTTCTTGT AGGCAAACGG TCCGTTTGCC
34751 34801 34851 34901 34951 35001	AGACACATCA CCTGGCTTCG GGACCGAAGC CATCCACCAC GTAGGTGGTG TGCGAGTCAC ACGCTCAGTG TTTTTTATTC AAAAAATAAG TGAACGCGCT ACTTGCGCGA GATAATGGCA CTATTACCGT	GGTTCTATGT CCAAGATACA CGCAGAATAA GCGTCTTATT ACACGGGAGG TGTGCCCTCC CAAAAGATTA GTTTTCTAAT CCCCTCCGGT GGGGAGGCCA TTTGTAAGAT AACATTCTA	ATAGGTGAGA AAACTCCTTC TTTGAGGAAG GCCACACCCA CGGTGTGGGT AGCGGGAAGA TCGCCCTTCT TCCAAAACCT AGGTTTTGGA GCCGTGGTCA CCGCACCAGT GTTGCACAAT CAACGTGTTA TAAAGGCTAA	ATGCGCCGCT TACGCGGCGA GCCAACCTAC CGGTTGGATG GCTGGAAGAA CGACCTTCTT CAAAATGAAG GTTTTACTTC AACTCTACAG TTGAGATGTC GGCTTCCAAA CCGAAGGTTT ACCCTTCAGG	GGTCCGCGGG GCCCTGATAA CGGGACTATT ACATTCGTTC TGTAAGCAAG CCATGTTTTT GGTACAAAAA ATCTATTAAG TAGATAATTC CCAAAGAACA GGTTTCTTGT AGGCAAACGG TCCGTTTGCC

Figure 26 AK

35151	CCACCTTCTC	AATATATCTC	TAAGCAAATC	CCGAATATTA	AGTCCGGCCA
33434		TTATATAGAG			
	GGIGGAAGAG	IMIMIMONG	ATTCGTTIAG	GGCTIATAAT	1CAGGCCGG1
35201		CTGCTCCAGA			
	AACATTTTTA	GACGAGGTCT	CGCGGGAGGT	GGAAGTCGGA	GTTCGTCGCT
35251	ATCATGATTG	CAAAAATTCA	GGTTCCTCAC	AGACCTGTAT	AAGATTCAAA
	TAGTACTAAC	GTTTTTAAGT	CCAAGGAGTG	TCTGGACATA	TTCTAAGTTT
25201	>0000X	TAACAAAAAT	*CCCCC***CC	CCTACCTCCC	TTCCCACCCC
35301		ATTGTTTTTA			
	TCGCCTTGTA	ATTGTTTTTA	TGGCGCTAGG	GCATCCAGGG	AAGCGICCCG
35351		TAATCGTGCA			
	GTCGACTTGT	ATTAGCACGT	CCAGACGTGC	CTGGTCGCGC	CGGTGAAGGG
					•
35401	CGCCAGGAAC	CATGACAAAA	GAACCCACAC	TGATTATGAC	ACGCATACTC
	GCGGTCCTTG	GTACTGTTTT	CTTGGGTGTG	ACTAATACTG	TGCGTATGAG
35451	CCACCTATCC	TAACCAGCGT	AGCCCCGATG	TAAGCTTGTT	GCATGGGCGG
33331		ATTGGTCGCA			
	ccrconinco	7111001000			
25501	CC200200222	TGCAAGGTGC	TO COTO A A A A A	ATCACCCAAA	CCCTCCCCCA
35501					
	GCTATATTTT	ACGTTCCACG	ACGAGITIII	TAGICCGITI	CGGMGCGCGI
35551		CACATCGTAG			
	TTTTTCTTTC	GTGTAGCATC	AGTACGAGTA	CGTCTATTTC	CGTCCATTCG
35601	TCCGGAACCA	CCACAGAAAA	AGACACCATT	TTTCTCTCAA	ACATGTCTGC
	AGGCCTTGGT	GGTGTCTTTT	TCTGTGGTAA	AAAGAGAGTT	TGTACAGACG
35651	GGGTTTCTGC	ATAAACACAA	AATAAAATAA	CAAAAAAACA	TTTAAACATT
••••		TATTTGTGTT			
	CCC/22.07.00			•	
35701	みぐみ みぐぐぐがぐか	CTTACAACAG	CAAAAACAAC	ССТТАТАВСС	ATAAGACGGA
33701		GAATGTTGTC			
	TCTTCGGACA	GAAIGIIGIC	CITITION	GGAATATICG	1711110001
		coodcoomes	CCCT> > > > > >	10000000000000000000000000000000000000	COCNOCNANA
35751		GCCGGCGTGA			
	GATGCCGGTA	CGGCCGCACT	GGCATTTTTT	TGACCAGTGG	CACTAATTT
35801	AGCACCACCG				
	TCGTGGTGGC	TGTCGAGGAG	CCAGTACAGG	CCTCAGTATT	ACATTCTGAG
35851	GGTAAACACA	TCAGGTTGAT	TCACATCGGT	CAGTGCTAAA	AAGCGACCGA
••••		AGTCCAACTA			
35001	AATAGCCCGG	CCCAATACAT	ACCCGC AGGC	GTAGAGACAA	CATTACAGCC
22301		CCCTTATGTA			
	1-1W1CGGGCC	CCCITAIGIA	1000001000	CHICICIGII	J11211.01C90
				010111101	C202222020
35951	CCCATAGGAG				
	GGGTATCCTC	CATATTGTTT	TAATTATCCT	CTCTTTTTGT	GTATTTGTGG
36001	TGAAAAACCC	TCCTGCCTAG	GCAAAATAGC	ACCCTCCCGC	TCCAGAACAA
	ACTTTTTGGG	AGGACGGATC	CGTTTTATCG	TGGGAGGGCG	AGGTCTTGTT

Figure 26 AL

36101	AAAGAAAACC	TATTAAAAAA	ACACCACTCG	ACACGGCACC	AGCTCAATCA
	TTTCTTTTGG	TTTTTTAATA	TGTGGTGAGC	TGTGCCGTGG	TCGAGTTAGT
36151	GTCACAGTGT	AAAAAAGGGC	CAAGTGCAGA	GCGAGTATAT	ATAGGACTAA
	CAGTGTCACA	TTTTTTCCCG	GTTCACGTCT	CGCTCATATA	TATCCTGATT
36201		AACGGTTAAA			
	TTTTACTGCA	TTGCCAATTT	CAGGTGTTTT	TTGTGGGTCT	TTTGGCGTGC
36251		CCCAGAAACG			
	GCTTGGATGC	GGGTCTTTGC	TTTCGGTTTT	TTGGGTGTTG	AAGGAGTTTA
36301		GTTTTCCCAC			
	GCAGTGAAGG	CAAAAGGGTG	CAATGCAGTG	AAGGGTAAAA	TTCTTTGAT
26251	6)) FFFG663)	CACATACAAG	mma cmcccccc	CT	CCTCXCCCC
36351		GTGTATGTTC			
	GTTAAGGGTT	GIGIAIGIIC	MATGAGGCGG	GATTITGGAT	GCAG1666CG
36401	CCCCTTCCCA	CGCCCGCGC	CACCTCACAA	ACTCCACCC	СТСАТТАТСА
20401		GCGGGGGGG			
	0000.2.0001		0.00		
			•		PacI
				•	~~~~~
36451	TATTGGCTTC	AATCCAAAAT	AAGGTATATT	ATTGATGATG	TTAATTAAGA
	ATAACCGAAG	TTAGGTTTTA	TTCCATATAA	TAACTACTAC	AATTAATTCT
36501		GCGACGCGAG			
	TAAGCCTAGA	CGCTGCGCTC	CGACCTACCG	GAAGGGGTAA	TACTAAGAAG
36551		CGGCATCGGG			
	AGCGAAGGCC	GCCGTAGCCC	TACGGGCGCA	ACGTCCGGTA	CGACAGGTCC
36601	C) CCT) C) TC	ACGACCATCA	CCCACACCMM	CNACCCCACC	AAAACCCCAC
30001		TGCTGGTAGT			
	GICCATCTAC	IGCIGGIAGI	CCCIGICGAA	GIICCGGICG	1111000010
36651	CAACCCTAAA	AAGGCCGCGT	Τ GCTGGCGTT	TTTCCATAGG	CTCCGCCCCC
20031		TTCCGGCGCA			
36701	CTGACGAGCA	TCACAAAAAT	CGACGCTCAA	GTCAGAGGTG	GCGAAACCCG
		AGTGTTTTTA			
36751	ACAGGACTAT	AAAGATACCA	GGCGTTTCCC	CCTGGAAGCT	CCCTCGTGCG
	TGTCCTGATA	TTTCTATGGT	CCGCAAAGGG	GGACCTTCGA	GGGAGCACGC
36801	CTCTCCTGTT				
	GAGAGGACAA	GGCTGGGACG	GCGAATGGCC	TATGGACAGG	CGGAAAGAGG
36851	CTTCGGGAAG				
	GAAGCCCTTC	GCACCGCGAA	AGAGTATCGA	GTGCGACATC	CATAGAGTCA
36901	TCGGTGTAGG	TCGTTCGCTC	CAAGCTGGGC		
		AGCAAGCGAG			MMAAAAAA

Figure 26 AM

37001	CGGTAAGACA	CGACTTATCG	CCACTGGCAG	CAGCCACTGG	TAACAGGATT
	GCCATTCTGT	GCTGAATAGC	GGTGACCGTC	GTCGGTGACC	ATTGTCCTAA
37051	AGCAGAGCGA	GGTATGTAGG	CGGTGCTACA	GAGTTCTTGA	AGTGGTGGCC
		CCATACATCC			
37101	TAACTACGGC	TACACTAGAA	GGACAGTATT	TGGTATCTGC	GCTCTGCTGA
		ATGTGATCTT			
37151	AGCCAGTTAC	CTTCGGAAAA	AGAGTTGGTA	GCTCTTGATC	CGGCAAACAA
• • • • • • • • • • • • • • • • • • • •		GAAGCCTTTT			
37201	ACCACCGCTG	GTAGCGGTGG	TTTTTTTGTT	TGCAAGCAGC	AGATTACGCG
		CATCGCCACC			
			•		
37251	CAGAAAAAA	GGATCTCAAG	AAGATCCTTT	GATCTTTTCT	ACGGGGTCTG
		CCTAGAGTTC			
37301	ACGCTCAGTG	GAACGAAAAC	TCACGTTAAG	GGATTTTGGT	CATGAGATTA
	TGCGAGTCAC	CTTGCTTTTG	AGTGCAATTC	CCTAAAACCA	GTACTCTAAT
37351	TCAAAAAGGA	TCTTCACCTA	GATCCTTTTA	AATCAATCTA	AAGTATATAT
	AGTTTTTCCT	AGAAGTGGAT	CTAGGAAAAT	TTAGTTAGAT	TTCATATATA
37401	GAGTAAACTT	GGTCTGACAG	TTACCAATGC	TTAATCAGTG	AGGCACCTAT
	CTCATTTGAA	CCAGACTGTC	AATGGTTACG	AATTAGTCAC	TCCGTGGATA
37451		TGTCTATTTC			
	GAGTCGCTAG	ACAGATAAAG	CAAGTAGGTA	TCAACGGACT	GAGGGGCAGC
37501		TACGATACGG			
	ACATCTATTG	ATGCTATGCC	CTCCCGAATG	GTAGACCGGG	GTCACGACGT
37551		GAGACCCACG			
	TACTATGGCG	CTCTGGGTGC	GAGTGGCCGA	GGTCTAAATA	GTCGTTATTT
		•			
37601		GGAAGGGCCG			
	GGTCGGTCGG	CCTTCCCGGC	TCGCGTCTTC	ACCAGGACGT	TGAAATAGGC
37651	CCTCCATCCA	GTCTATTAAT	TGTTGCCGGG	AAGCTAGAGT	AAGTAGTTCG
	GGAGGTAGGT	CAGATAATTA	ACAACGGCCC	TTCGATCTCA	TTCATCAAGC
37701		GTTTGCGCAA			
	GGTCAATTAT	CAAACGCGTT	GCAACAACGG	TAACGATGTC	CGTAGCACCA
37751	GTCACGCTCG	TCGTTTGGTA	TGGCTTCATT	CAGCTCCGGT	TCCCAACGAT
	CAGTGCGAGC	AGCAAACCAT	ACCGAAGTAA	GTCGAGGCCA	AGGGTTGCTA
					00000
37801		TACATGATCC			
	GTTCCGCTCA	ATGTACTAGG	GGGTACAACA	CGTTTTTTCG	CCAATCGAGG
				mm0000000	mcmmamca cm
37851		CGATCGTTGT			
	AAGCCAGGAG	GCTAGCAACA	GTCTTCATTC	AACCGGCGTC	ACAATAGTGA

Figure 26 AN

37951	GATGCTTTTC	TGTGACTGGT	GAGTACTCAA	CCAAGTCATT	CTGAGAATAG
		ACACTGACCA			
38001	TGTATGCGGC				
	ACATACGCCG	CTGGCTCAAC	GAGAACGGGC	CGCAGTTGTG	CCCTATTATG
20051		AGCAGAACTT	#	CATCATTCCA	እ እ እ CC ጥጥርጥጥ
38051		TCGTCTTGAA			
	GCGCGGIGIA	regrerren	ATTITICACOA	011.01/2.001	11100.110.1
38101	CGGGGCGAAA	ACTCTCAAGG	ATCTTACCGC	TGTTGAGATC	CAGTTCGATG
		TGAGAGTTCC			
38151	TAACCCACTC				
	ATTGGGTGAG	CACGTGGGTT	GACTAGAAGT	CGTAGAAAAT	GAAAGTGGTC
38201	CCTTTCTCCC	TGAGCAAAAA	CAGGAAGGCA	AAATGCCGCA	AAAAAGGGAA
38201		ACTCGTTTTT			
	00.22.0000				
38251	TAAGGGCGAC				
	ATTCCCGCTG	TGCCTTTACA	ACTTATGAGT	ATGAGAAGGA	AAAAGTTATA
				>mc>ccc>x	3 C 3 T 3 T T T T T C 3
38301		TTTATCAGGG AAATAGTCCC			
	ATAACTICGI	AAATAGTCCC	AATAACAGAG	IACICOCCIA	.0.77.22.0.
38351	ATGTATTTAG	AAAAATAAAC	AAATAGGGGT	TCCGCGCACA	TTTCCCCGAA
		TTTTTATTTG			
38401	AAGTGCCACC				
	TTCACGGTGG	ACTGCAGATT	CTTTGGTAAT	AATAGTACTG	TAATTGGATA
20/51	AAAAATAGGC	GTATCACGAG	CCCCTTTCGT	CTTCAAGAAT	TGGATCCGAA
30431		CATAGTGCTC			
	,	PacI			
				20)	
38501	TTCTTAATTT				
	AAGAATTAAA	GAATTAATT	(2FO ID NO	: 331	

Figure 26 AO

WO 02/022080

1				GAAGCCAATA CTTCGGTTAT	
51				TGGGAACGGG ACCCTTGCCC	
101	TAGTAGTGTG	GCGGAAGTGT	GATGTTGCAA	GTGTGGCGGA	ACACATGTAA
151				CACACCGCCT GTGTGCGCCG	
131	CGCTGCCTAC	ACCGTTTTCA	CTGCAAAAAC	CACACGCGGC	CACATGTGTC
201				GATGTTGTAG CTACAACATC	
251				GGGAAAACTG CCCTTTTGAC	
301				TAGCGCGTAA ATCGCGCATT	
351				AGACTCGCCC TCTGAGCGGG	
401				TTGGCGTTTT AACCGCAAAA	
451	GCGGCCGCGA	TCCATTGCAT	ACGTTGTATC	CATATCATAA	TATGTACATT
501	TATATTGGCT	CATGTCCAAC	ATTACCGCCA	GTATAGTATT	GATTATTGAC
551	•			ACAACTGTAA ATTAGTTCAT	
551	ATCAATAATT	ATCATTAGTT	AATGCCCCAG	TAATCAAGTA	TCGGGTATAT
601				ATGGCCCGCC TACCGGGCGG	
651	CCCAACGACC GGGTTGCTGG	CCCGCCCATT GGGCGGGTAA	GACGTCAATA CTGCAGTTAT	ATGACGTATG TACTGCATAC	TTCCCATAGT AAGGGTATCA
701	AACGCCAATA TTGCGGTTAT	GGGACTTTCC CCCTGAAAGG	ATTGACGTCA TAACTGCAGT	ATGGGTGGAG TACCCACCTC	TATTTACGGT ATAAATGCCA
751	AAACTGCCCA	CTTGGCAGTA	CATCAAGTGT	ATCATATGCC TAGTATACGG	AAGTACGCCC TTCATGCGGG
801	CCTATTGACG	TCAATGACGG	TAAATGGCCC	GCCTGGCATT	ATGCCCAGTA
	GGATAACTGC	AGTTACTGCC	ATTTACCGGG	CGGACCGTAA	TACGGGTCAT

Figure 27A

				\	
901	TCGCTATTAC	CATGGTGATG	CGGTTTTGGC	AGTACATCAA	TGGGCGTGGA
		GTACCACTAC			-
	700011172110	0111001101110			ccocncci
951		ACTCACGGGG			
	ATCGCCAAAC	TGAGTGCCCC	TAAAGGTTCA	GAGGTGGGGT	AACTGCAGTT
1001	TGGGAGTTTG	TTTTGGCACC	AAAATCAACG	GGACTTTCCA	AAATGTCGTA
	ACCCTCAAAC	AAAACCGTGG	ግተተተን የተተ ተረር	CCTGAAAGGT	TTTACAGCAT
1051	> 0 > > CMCCCCC	CCCATTGACG	C)	CEN COCCECTO	NOCOMOCONO.
1051					
	TGTTGAGGCG	GGGTAACTGC	GTTTACCCGC	CATCCGCACA	TGCCACCCTC
1101	GTCTATATAA	GCAGAGCTCG	TTTAGTGAAC	CGTCAGATCG	CCTGGAGACG
	CAGATATATT	CGTCTCGAGC	AAATCACTTG	GCAGTCTAGC	GGACCTCTGC
1151	CCATCCACGC	TGTTTTGACC	TCCATAGAAG	ACACCGGGAC	CGATCCAGCC
		ACAAAACTGG			
	001A0010C0	nemphic 100		.0.0000010	00111001000
1001	managanana	0011000000	30000033CCC	CCAMMCCCCC	TGCCAAGAGT .
1201					
	AGGCGCCGGC	CCTTGCCACG	TAACCTTGCG	CCTAAGGGGC	ACGGTTCTCA
1251		ACCATGGCCG			
	CTCTAGACGG	TGGTACCGGC	CGTTCACCAG	GTTCTCCAGG	CACGGGCCGA
1301	GGTCCACCGT	GAGGGAGAGG	ATGAGGAGGG	CCGAGCCCGC	CGCCGACAGG
		CTCCCTCTCC			
		0.000.0.00			
1251	CMC> CC> CC>	CCGAGCCCGC	CCCACECCCC	CACCCCCCC	TOTOCACCOA
1351					
	CACTCCTCCT	GGCTCGGGCG	GCGTCACCCG	CACCCGCGCGC	ACAGGTCCCT
1401	• • • • • • • • • • • • • • • • • • • •	CACGGCGCCA			
	GGACCTCTTC	GTGCCGCGGT	AGTGGAGGAG	GTTGTGGCGG	CGGTGGTTGC
1451	CCGACTGCGC	CTGGCTGGAG	GCCCAGGAGG	ACGAGGAGGT	GGGCTTCCCC
		GACCGACCTC			
	••••	•			
1501	CTCACCCCC	AGGTGCCCCT	GACCCCCATG	ACCTACAAGG	CCCCCCTCCA
1201					
	CACTCCGGGG	TCCACGGGGA	CICCGGGIAC	TOGATOTICC	CGCGGCACCI
1551	CCTGTCCCAC				
	GGACAGGGTG	AAGGACTTCC	TCTTCCCGCC	GGACCTCCCG	GACTAGGTGA
1601	CCCAGAAGAG	GCAGGACATC	CTGGACCTGT	GGGTGTACCA	CACCCAGGGC
		CGTCCTGTAG			
		••••			
1651	TACTTCCCCG	ACTICION CANA	CTACACCCC	GCCCCCCC.	TCAGGTTCCC
1001		TGACCGTCTT			
	ATGAAGGGGC	TGACCGTCTT	GWIGIGGGG	CCGGGGCCG1	AG 1 C CANGGG
					0000101100
1701	CCTGACCTTC				
	GGACTGGAAG	CCGACCACGA	AGTTCGACCA	CGGGCACCTC	GGGCTCTTCC
1751	TGGAGGAGGC	CAACGAGGGC	GAGAACAACT	GCGCCGCCCA	CCCCATGTCC
-		GTTGCTCCCG			

Figure 27B

1851	CTCCAAGCTG	GCCTTCCACC	ACGTGGCCAG	GGAGCTGCAC	CCCGAGTACT
	GAGGTTCGAC	CGGAAGGTGG	TGCACCGGTC	CCTCGACGTG	GGGCTCATGA
1901	ACAAGGACTG	CTAAAGCCCG	GGCAGATCTG	СТСТСССТТС	TAGTTGCCAG
1701		GATTTCGGGC	• • • • • • •		
	TGTTCCTGAC	GATTICGGGC	CCGICIAGAC	GACACGGAAG	ATCAACGGTC
					maa aamaa
1951		TTTGCCCCTC			
	GGTAGACAAC	AAACGGGGAG	GGGGCACGGA	AGGAACTGGG	ACCTTCCACG
2001		GTCCTTTCCT			
	GTGAGGGTGA	CAGGAAAGGA	TTATTTTACT	CCTTTAACGT	AGCGTAACAG
2051	TGAGTAGGTG	TCATTCTATT	CTGGGGGGTG	GGGTGGGGCA	GGACAGCAAG
	ACTCATCCAC	AGTAAGATAA	GACCCCCCAC	CCCACCCCGT	CCTGTCGTTC
2101	CCCCACCATT	GGGAAGACAA	TAGCAGGCAT	GCTGGGGATG	CGGTGGGCTC
2101		CCCTTCTGTT			
	CCCCTCCTAN	ccciicidii	AICOICCOIA	CONCCCCIAC	000110000110
2151	m>mcccccnm	CGGCGCGCCG	መስርጥር እ እ አጥር	TOTO COCOTO	CCTTAACCCT
2131					
	ATACCGGCTA	GCCGCGCGGC	ATGACTITAC	ACACCCGCAC	CGAATICCCA
2201	•	ATATAAGGTG			
	CCCTTTCTTA	TATATTCCAC	CCCCAGAATA	CATCAAAACA	TAGACAAAAC
2251	•••••	CGCCGCCATG		and the second s	
	GTCGTCGGCG	GCGGCGGTAC	TCGTGGTTGA	GCAAACTACC	TTCGTAACAC
				•	
2301	AGCTCATATT	TGACAACGCG	CATGCCCCCA	TGGGCCGGGG	TGCGTCAGAA
	TCGAGTATAA	ACTGTTGCGC	GTACGGGGGT	ACCCGGCCCC	ACGCAGTCTT
			,		
2351	TGTGATGGGC	TCCAGCATTG	ATGGTCGCCC	CGTCCTGCCC	GCAAACTCTA
	ACACTACCCG	AGGTCGTAAC	TACCAGCGGG	GCAGGACGGG	CGTTTGAGAT
2401	СТАССТТСАС	CTACGAGACC	GTGTCTGGAA	CGCCGTTGGA	GACTGCAGCC
2401		GATGCTCTGG			
	dwiddwicid	QU'OCICIOO	chenomeer	00000.2.001	0.00.00000
2451	TOCOCOCOC	CTTCAGCCGC	TOTACOCACO	CCCCCCCCCA	TTCTCACTCA
2451		GAAGTCGGCG			
	AGGCGGCGGC	GAAGTCGGCG	ACGICGGIGG	CGGGCGCCCI	MACACIGACI
			mmoc	maa. 00mm00	OCOMO NOCOC
2501	CTTTGCTTTC				
	GAAACGAAAG	GACTCGGGCG	AACGTTTGTC	ACGTCGAAGG	GCAAGTAGGC
2551	CCCGCGATGA				
	GGGCGCTACT	GTTCAACTGC	CGAGAAAACC	GTGTTAACCT	AAGAAACTGG
		•			
2601	CGGGAACTTA				
	GCCCTTGAAT	TACAGCAAAG	AGTCGTCGAC	AACCTAGACG	CGGTCGTCCA
2651	TTCTGCCCTG	AAGGCTTCCT	CCCCTCCCAA	TGCGGTTTAA	AACATAAATA
		TTCCGAAGGA			
2701	AAAAACCAGA	СФСФСФФФФСС	ATTTGGATCA	AGCAAGTGTC	TTGCTGTCTT
2,01	#######CCVGV	GAGACAAACC	ТАДАССТАСТ	TCGTTCACAC	AACGACAGAA
	1111100101	- ANGREAMEC	-marce tugi		

Figure 27C

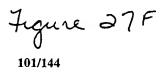
WO 02/022	080				PCT/US01/28861
2751	TATT	TTTTGCGCGC	GCGGTAGGCC	CGGGACCAGC	TCTCGGTC
				GCCCTGGTCG	
2801	GTTGAGGGTC				
				CACCATTTCC	
2851	TGTTCAGATA				
				ACCCCACCTC	
2901	TGCAGAGCTT				
				ATCTACTAGG	
2951	GGAGCGCTGG			AAAGTCATCG	
2001	CCAGGGGCAG				
3001				GTTTCGCCAA	
2051	GGGTGCATAC				
3051				AACCTGACAT	
			•		
3101	GGCTATGTTC			TAAGTACAAC	
3151	CCAGCACAGT			TAAACAGTAC	
3201	GGAAATGCGT			AACACTGGAG	
3251	CATGCATTCG			GGGTGCCCGC	
3301	CGAAGATATT	TCTGGGATCA	CTAACGTCAT	TCAACACAAG	CAGGATGAGA
3351	TCGTCATAGG	CCATTTTTAC	TTTCCCCCCC	CGGAGGGTGC GCCTCCCACG	GTCTGACGCC
3401	TATAATGGTT	CCATCCGGCC	CAGGGGGCTA	CAATGGGAGT	GTCTAAACGT
3451	TTTCCCACGC			AGTACAGATG	
3501	ATGAAGAAAA	CGGTTTCCGG	GGTAGGGGAG	ATCAGCTGGG	AAGAAAGCAG
		•		TAGTCGACCC	
3551	GTTCCTGAGC	AGCTGCGACT	TACCGCAGCC	GGTGGGCCCG	TAAATCACAC
				CCACCCGGGC	
3601	CTATTACCGG				
				TCGACGTCGA	
3651	CTGAGCAGGG				
	GACTCGTCCC	CCCGGTGAAG	CAATTCGTAC	AGGGACTGAG	CGTACAAAAG

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3701		TCCGCCAGAA	·		
	GGACTGGTTT	AGGCGGTCTT	CCGCGAGCGG	CGGGTCGCTA	TCGTCAAGAA
3751	GCAAGGAAGC	AAAGTTTTTC	AACGGTTTGA	GACCGTCCGC	CGTAGGCATG
		TTTCAAAAAG			
	CGIICCIICG	1110/2022/10	· · · · · · · · · · · · · · · · · · ·	CIOGCAGGCG	OCHICCOIAC
3801	CTTTTGAGCG	TTTGACCAAG	CAGTTCCAGG	CGGTCCCACA	GCTCGGTCAC
	GAAAACTCGC	AAACTGGTTC	GTCAAGGTCC	GCCAGGGTGT	CGAGCCAGTG
2051	CMCCMCMACC	GCATCTCGAT	CCACCATATC	ጥርርጥርርጥጥጥር	CCCCCTTCCC
3851	••••				
	GACGAGATGC	CGTAGAGCTA	GGTCGTATAG	AGGAGCAAAG	UGCCCAACCC
3901	GCGGCTTTCG	CTGTACGGCA	GTAGTCGGTG	CTCGTCCAGA	CGGGCCAGGG
	CGCCGAAAGC	GACATGCCGT	CATCAGCCAC	GAGCAGGTCT	GCCCGGTCCC
				•	
3951	TCATGTCTTT	CCACGGGCGC	AGGGTCCTCG	TCAGCGTAGT	CTGGGTCACG
	AGTACAGAAA	GGTGCCCGCG	TCCCAGGAGC	AGTCGCATCA	GACCCAGTGC
4001	CTCNACCCCT	GCGCTCCGGG	רדוברברברדוב	GCCAGGGTGC	CCTTCACCCT
4001		CGCGAGGCCC			
	CACTICCCA	CGCGAGGCCC	GACGCGCGAC	CGGICCCACG	COARCICCOA
4051	GGTCCTGCTG	GTGCTGAAGC	GCTGCCGGTC	TTCGCCCTGC	GCGTCGGCCA
	CCAGGACGAC	CACGACTTCG	CGACGGCCAG	AAGCGGGACG	CGCAGCCGGT
4101		GACCATGGTG			
	CCATCGTAAA	CTGGTACCAC	AGTATCAGGT	CGGGGAGGCG	CCGCACCGGG
4151		GCTTGCCCTT			
	AACCGCGCGT	CGAACGGGAA	CCTCCTCCGC	GGCGTGCTCC	CCGTCACGTC
4201	እ ር ጥጥጥጥር እ ርር	GCGTAGAGCT	TECCCCCCAC	אמשמעמעמעמעמע	тесесесьст
4201		CGCATCTCGA			
	TGAAAACTCC	CGCATCTCGA	ACCCGCGCTC	IIIAIGGCIA	AGGCCCTCA
4251	AGGCATCCGC	GCCGCAGGCC	CCGCAGACGG	TCTCGCATTC	CACGAGCCAG.
		CGGCGTCCGG			
4301		GCCGTTCGGG			
	CACTCGAGAC	CGGCAAGCCC	CAGTTTTTGG	TCCAAAGGGG	GTACGAAAAA
4351	a. =a.c.===	mm. 00m0m00	mmmac. mc. c	COCOMORCO	CCCTCCCTCA
4351	GATGCGTTTC				
	CTACGCAAAG	AATGGAGACC	AAAGGTACTC	GGCCACAGGT	GUGAGUUACT
4401	CGAAAAGGCT	CTCCCTCTCC	СССТАТАСАС	ACTTGAGAGG	CCTGTCCTCG
4401		CAGGCACAGG			
	GCTTTTCCGA	CAGGCACAGG	GGCATATGTC	IGAACICICC	GGACAGGAGC
4451	AGCGGTGTTC	CGCGGTCCTC	CTCGTATAGA	AACTCGGACC	ACTCTGAGAC
		GCGCCAGGAG			
4501	AAAGGCTCGC				
	TTTCCGAGCG	CAGGTCCGGT	CGTGCTTCCT	CCGATTCACC	CTCCCCATCG
4551	GGTCGTTGTC				
	CCAGCAACAG	GTGATCCCCC	AGGTGAGCGA	GGTCCCACAC	TTCTGTGTAC
					mama acces c
4601	TCGCCCTCTT				
	AGCGGGAGAA	GCCGTAGTTC	CTTCCACTAA	CCAAACATCC	ACATCCGGTG

Figure 27E

4701		CTCTTCCGCA GAGAAGGCGT		
4751	•	TCTGAAAAGC AGACTTTTCG		
4801		GAGGAGGATT		
4851		CTCCTCCTAA		
4002	· -	GCGTAGGTAG		
4901		CAAACGACCC GTTTGCTGGG	 	
4951		GTTTGGTTTT CAAACCAAAA		
5001		CACGTATTCG GTGCATAAGC		
5051		CGTCGGGCAC GCAGCCCGTG		
5101		TCAACGCTGG		
		AGTTGCGACC		
5151		CCCCCCCCC		
5201		CGTCCGGGGG GCAGGCCCCC		
5251		TCGAAGTAGT AGCTTCATCA		
5301		GCGGGCGCA CGCCCGCCGT		
5351	CCCCATGGCA	TGGGGTGGGT ACCCCACCCA		
5401	GTAAACGTAG			
		TCCCCGAGAG		
5451	TTCCACCGCG AAGGTGGCGC	CTACGACCGC		
5501				CTGCTCGGAA GACGAGCCTT
5551				GTTGGACGCT CAACCTGCGA



5651	GAGGCGTAGG	AGTCGCGCAG	CTTGTTGACC	AGCTCGGCGG	TGACCTGCAC
	CTCCGCATCC	TCAGCGCGTC	GAACAACTGG	TCGAGCCGCC	ACTGGACGTG
5701	GTCTAGGGCG	CAGTAGTCCA	GGGTTTCCTT	GATGATGTCA	TACTTATCCT
3,01				CTACTACAGT	
	CAGAICCCGC	GICAICAGGI	CCCMMOON	o inc incho!	7110/211110011
E761		中中中へことととこ	TO COCOTTO	GGACAAACTC	ייירכרכביריריי
5751				CCTGTTTGAG	
	CAGGGAAAAA	AAAGGTGTCG	AGCGCCAACI	CCIGIIIGAG	ANGCGCCAGA
			****	CCCTCCCAAC	CCMXXCXCCC
5801				GCCTCCGAAC	
	AAGGTCATGA	GAACCTAGCC	TTTGGGCAGC	CGGAGGCTTG	CCATTCTCGG
				000000000	OCCUMPATOR CETA
5851				GGCGCAGCAT	
	ATCGTACATC	TTGACCAACT	GCCGGACCAT	CCGCGTCGTA	GGGAAAAGAT
				001000100	omacomes.cc
5901				GGAGCGAGGT	
	GCCCATCGCG	CATACGGACG	CGCCGGAAGG	CCTCGCTCCA	CACCCACTCG
5951				TACTGGTATT	
	CGTTTCCACA	GGGACTGGTA	CTGAAACTCC	ATGACCATAA	ACTTCAGTCA
6001				AAAGTCCGTG	
	CAGCAGCGTA	GGCGGGACGA	GGGTCTCGTT	TTTCAGGCAC	GCGAAAAACC
6051				CGTTGAAGAG	
	TTGCGCCTAA	ACCGTCCCGC	TTCCACTGTA	GCAACTTCTC	ATAGAAAGGG
6101				AAGGGTCCCG	
	CGCGCTCCGT	ATTTCAACGC	ACACTACGCC	TTCCCAGGGC	CGTGGAGCCT
6151				GATCTCGTCA	
	TGCCAACAAT	TAATGGACCC	GCCGCTCGTG	CTAGAGCAGT	TTCGGCAACT
	•				•
6201				AGCGCGGGAT	
	ACAACACCGG	GTGTTACATT	TCAAGGTTCT	TCGCGCCCTA	CGGGAACTAC
6251				AGCTCTTCAG	
	CTTCCGTTAA	AAAATTCAAG	GAGCATCCAC	TCGAGAAGTC	CCCTCGACTC
6301	CCCGTGCTCT	GAAAGGGCCC	AGTCTGCAAG	ATGAGGGTTG	GAAGCGACGA
	GGGCACGAGA	CTTTCCCGGG	TCAGACGTTC	TACTCCCAAC	CTTCGCTGCT
				•	
6351	ATGAGCTCCA	CAGGTCACGG	GCCATTAGCA	TTTGCAGGTG	GTCGCGAAAG
• • • •	TACTCGAGGT	GTCCAGTGCC	CGGTAATCGT	AAACGTCCAC	CAGCGCTTTC
					•
6401	GTCCTAAACT	GGCGACCTAT	GGCCATTTTT	TCTGGGGTGA	TGCAGTAGAA
••••	CAGGATTTGA	CCGCTGGATA	CCGGTAAAAA	AGACCCCACT	ACGTCATCTT
6451	GGTAAGCGGG	TCTTGTTCCC	AGCGGTCCCA	TCCAAGGTTC	GCGGCTAGGT
0471	CCATTCGCCC	AGAACAAGGG	TCGCCAGGGT	AGGTTCCAAG	CGCCGATCCA
6501	CTCGCGCGGC	AGTCACTAGA	GGCTCATCTC	CGCCGAACTT	CATGACCAGC
0501	GAGCGCGCCC	TCAGTGATCT	CCGAGTAGAG	GCGGCTTGAA	GTACTGGTCG

Figure. 276



6601	TACATCGTAG	GTGACAAAGA	GACGCTCGGT	GCGAGGATGC	GAGCCGATCG
	ATGTAGCATC	CACTGTTTCT	CTGCGAGCCA	CGCTCCTACG	CTCGGCTAGC
6651		GATCTCCCGC			
	CCTTCTTGAC	CTAGAGGGCG	GTGGTTAACC	TCCTCACCGA	TAACTACACC
6701	TGAAAGTAGA	AGTCCCTGCG	ACGGGCCGAA	CACTCGTGCT	GGCTTTTGTA
	ACTTTCATCT	TCAGGGACGC	TGCCCGGCTT	GTGAGCACGA	CCGAAAACAT
6751	AAAACGTGCG	CAGTACTGGC	AGCGGTGCAC	GGGCTGTACA	TCCTGCACGA
		GTCATGACCG			
6801	GGTTGACCTG				
		TGCTGGCGCG			
6851	TCGCCTGGCG	GGTTTGGCTG	GTGGTCTTCT	ACTTCGGCTG	CTTGTCCTTG
	AGCGGACCGC	CCAAACCGAC	CACCAGAAGA	TGAAGCCGAC	GAACAGGAAC
6901		TGCTCGAGGG			
	TGGCAGACCG	ACGAGCTCCC	CTCAATGCCA	CCTAGCCTOG	TGGTGCGGCG
6951		AGTCCAGATG			
	CGCTCGGGTT	TCAGGTCTAC	AGGCGCGCGC	CGCCAGCCTC	GAACTACTGT
7001	ACATCGCGCA	GATGGGAGCT	GTCCATGGTC	TGGAGCTCCC	GCGGCGTCAG
		CTACCCTCGA			
7051	•	AGCTCCTGCA			
	CAGTCCGCCC	TCGAGGACGT	CCAAATGGAG	CGTATCTGCC	CAGTCCCGCG
7101	GGGCTAGATC	CAGGTGATAC	CTAATTTCCA	GGGGCTGGTT	GGTGGCGGCG
		GTCCACTATG			
7151		GCAAGAGGCC			
		CGTTCTCCGG			
7201		TGGGCCGCGG			
		ACCCGGCGCC			
7251	GTGACGCGGG	CGAGCCCCCG	GAGGTAGGGG	GGGCTCCGGA	CCCGCCGGGA
		GCTCGGGGGC			
7301	GAGGGGGCAG				
		CCCGTGCAGC			
7351	GCGCGTAGGT				
		ACGACCGCTT			
7401	CTGGCGCCTC				
		ACGCACTTCT			
7451	AGAGTTCGAC				
	TCTCAAGCTG	TCTTAGTTAA	AGCCACAGCA	ACTGCCGCCG	GACCGCGTTT

Figure 27H

7551	CTGCTCGATC	TCTTCCTCCT	GGAGATCTCC	GCGTCCGGCT	CGCTCCACGG
, , , ,		AGAAGGAGGA			
	GACGAGCIAG	AGAAGGAGGA	CCICIAGAGG	COCHOOCCOR	GCGNGGIGCC
7601	TGGCGGCGAG	GTCGTTGGAA	ATGCGGGCCA	TGAGCTGCGA	GAAGGCGTTG
	ACCGCCGCTC	CAGCAACCTT	TACGCCCGGT	ACTCGACGCT	CTTCCGCAAC
		•			
				***********	CMMCCCCNMC
7651		CGTTCCAGAC			
	TCCGGAGGGA	GCAAGGTCTG	CGCCGACATC	TGGTGCGGGG	GAAGCCGTAG
7701	פרפנינרפרפר	ATGACCACCT	GCGCGAGATT	GAGCTCCACG	TGCCGGGCGA
,,,,		TACTGGTGGA			
•	Caccacaca	TACTGGTGGA	CGCGCICIAA	CICGNOGIGC	ACGGCCCGCI
7751	AGACGGCGTA	GTTTCGCAGG	CGCTGAAAGA	GGTAGTTGAG	GGTGGTGGCG
	TCTGCCGCAT	CAAAGCGTCC	GCGACTTTCT	CCATCAACTC	CCACCACCGC
	.0.0000	•			
~~~		CCACGAAGAA	CD2C2D22CC	CACCCTCCCA	አርርጥርርእጥጥር
7801					
	CACACAAGAC	GGTGCTTCTT	CATGTATTGG	GTCGCAGCGT	TGCACCTAAG
7851	CTTCATATCC	CCCAAGGCCT	CAAGGCGCTC	CATGGCCTCG	TAGAAGTCCA
,031		GGGTTCCGGA			
	CAACTATAGG	GGGIICCGGA	GIICCGCGAG	GIACCOGNOC	
7901		GAAAAACTGG			
	GCCGCTTCAA	CTTTTTGACC	CTCAACGCGC	GGCTGTGCCA	ATTGAGGAGG
7051	mccacaacac	GGATGAGCTC	CCCCACAGTG	TCCCCCACCT	CCCCCTCAAA
7951					
	AGGTCTTCTG	CCTACTCGAG	CCGCTGTCAC	AGCGCGTGGA	GCGCGAGIII
8001	GGCTACAGGG	GCCTCTTCTT	CTTCTTCAAT	CTCCTCTTCC	ATAAGGGCCT
		CGGAGAAGAA			
	CCGNIGICCC	000.0,2.0,2.	0.2.0.2.0		
				0.0000000	>000000000
8051		TTCTTCTGGC			
	GGGGAAGAAG	AAGAAGACCG	CCGCCACCCC	CTCCCCCCTG	TGCCGCCGCT
8101	CCACCCCCA	CCGGGAGGCG	GTCGACAAAG	CGCTCGATCA	TCTCCCCGCG
0101		GCCCTCCGC			
	GCTGCCGCGT	GGCCCTCCGC	CAGCIGITIC	GCGAGCIAGI	HONOGOGGG
8151		ATGGTCTCGG			
	CGCTGCCGCG	TACCAGAGCC	ACTGCCGCGC	CGGCAAGAGC	GCCCCCGCGT
	••••				
0001	GTTGGAAGAC	COCCCCCCCCC	N TOTO COCOT	TATE CONTROL	CCCCCCCCCTC
8201	GTTGGAAGAC	GCCGCCGTC	AIGICCCGGI	1810001100	0000000000
	CAACCTTCTG	CGGCGGGCAG	TACAGGGCCA	ATACCCAACC	GCCCCCCGAC
8251	CCATGCGGCA	GGGATACGGC	GCTAACGATG	CATCTCAACA	ATTGTTGTGT
0202	CCTACGCCGT	CCCTATGCCG	ССАТТССТАС	GTAGAGTTGT	TAACAACACA
	GOINCOCCGI	CCCIMIGCOG			
					1000015000
8301	AGGTACTCCG	CCGCCGAGGG	ACCTGAGCGA	GTCCGCATCG	ACCGGATCGG
	TCCATGAGGC	GGCGGCTCCC	TGGACTCGCT	CAGGCGTAGC	TGGCCTAGCC
		03.0333.0000	MCM3 5 CC3 CM	<b>しかしかごかいごしか</b>	AGGTAGGCTG
8351	AAAACCTCTC	GAGAAAGGCG	1CIAACCAGI	CACAGICGCA	DOODATOOLIO
•	TTTTGGAGAG	CTCTTTCCGC	AGATTGGTCA	GTGTCAGCGT	TUCATUUGAU
8401	AGCACCGTGG	CGGGCGGCAG	CGGGCGGCGG	TCGGGGTTGT	TTCTGGCGGA
	TCCTCCCACC	CCCCCCCCTC	GCCCGCCGCC	AGCCCCAACA	AAGACCGCCT
	1CG1GGCVCC				

Figure 27I

•					
8501	TCGACAGAAG	CACCATGTCC	TTGGGTCCGG	CCTGCTGAAT	GCGCAGGCGG
		GTGGTACAGG			
	AGC:O:C::C	0.00			
8551		CCCAGGCTTC			
	AGCCGGTACG	GGGTCCGAAG	CAAAACTGTA	GCCGCGTCCA	GAAACATCAT
8601	GTCTTGCATG	AGCCTTTCTA	CCGGCACTTC	TTCTTCTCCT	TCCTCTTGTC
•		TCGGAAAGAT			
	CAGAACGIAC	10000010711	000001012.0		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,
				0000003.CDD	mcccccmxcc
8651		TGCATCTATC			
	GACGTAGAGA	ACGTAGATAG	CGACGCCGCC	GCCGCCTCAA	ACCGGCATCC
8701	TGGCGCCCTC	TTCCTCCCAT	GCGTGTGACC	CCGAAGCCCC	TCATCGGCTG
	ACCGCGGGAG	AAGGAGGGTA	CGCACACTGG	GGCTTCGGGG	AGTAGCCGAC
			• • • • • • • • • • • • • • • • • • • •		
8751	* * CC * CC CC T	AGGTCGGCGA	CAACGCGCTC	CCCTAATATC	CCTCCTCCA
8/31					
	TTCGTCCCGA	TCCAGCCGCT	GTTGCGCGAG	CCGATTATAC	CGGACGACG1
			•		
8801		GGTAGACTGG			
	GGACGCACTC	CCATCTGACC	TTCAGTAGGT	ACAGGTGTTT	CGCCACCATA
8851	GCGCCCGTGT	TGATGGTGTA	AGTGCAGTTG	GCCATAACGG	ACCAGTTAAC
0031		ACTACCACAT			
	CGCGGGCACA	ACIACCACAI	TCACGICATO		
				CON COMENCA	CCCC N C TO N N C
8901		CCCGGCTGCG			
	CCAGACCACT	GGGCCGACGC	TCTCGAGCCA	CATGGACTCT	GCGCTCATTC
8951	CCCTCGAGTC	AAATACGTAG	TCGTTGCAAG	TCCGCACCAG	GTACTGGTAT
	GGGAGCTCAG	TTTATGCATC	AGCAACGTTC	AGGCGTGGTC	CATGACCATA
	000				
0001	CCCACCAAAA	AGTGCGGCGG	CCCCTCCCC	TAGAGGGGCC	AGCGTAGGGT
9001		TCACGCCGCC			
	GGGTGGTTTT	TCACGCCGCC	GCCGACCGCC	ATCTCCCGG	ICGCAICCCA
9051	GGCCGGGGCT	CCGGGGGCGA	GATCTTCCAA	CATAAGGCGA	TGATATCCGT
	CCGGCCCCGA	GGCCCCCGCT	CTAGAAGGTT	GTATTCCGCT	ACTATAGGCA
	•				
9101	AGATGTACCT	GGACATCCAG	GTGATGCCGG	CGGCGGTGGT	GGAGGCGCGC
2202		CCTGTAGGTC			
	ICIACAIGGA	CCIGIAGGIC	CACIACOCC		•••••
			COLON DODO	CCCACCCCCA	N N N N C TC C TC
9151	GGAAAGTCGC	GGACGCGGTT	CCAGATGTTG	CGCAGCGGCA	AAAAGTGCTC
	CCTTTCAGCG	CCTGCGCCAA	GGTCTACAAC	GCGTCGCCGT	TTTTCACGAG
9201	CATGGTCGGG	ACGCTCTGGC	CGGTCAGGCG	CGCGCAATCG	TTGACGCTCT
	GTACCAGCCC	TGCGAGACCG	GCCAGTCCGC	GCGCGTTAGC	AACTGCGAGA
		••••			
0251	303000000	*******	ርጥርጥል አርርርር	<b>ር</b> ርልርጥርጥጥርር	GTGGTCTGGT
372T	ACACCGTGCA	ANNOUNDADA	CIGINAGCOG	CCTCACAACC	CACCAGACCA
	TCTGGCACGT	TTTCCTCTCG	GACATICGCC	COTOWOWNOR	CACCAGACCA
	•				
9301	GGATAAATTC	GCAAGGGTAT	CATGGCGGAC	GACCGGGGTT	CGAGCCCCGT
	CCTATTTAAG	CGTTCCCATA	GTACCGCCTG	CTGGCCCCAA	GCTCGGGGCA
9351	ATCCGCCCGT	CCGCCGTGAT	CCATGCGGTT	ACCGCCCGCG	TGTCGAACCC
100	MACCCCCCA	CCCCCCACTA	GGTACGCCAA	TEECEGECEC	ACAGCTTGGG
	TWOOFFGGGW	GGCGGCACIA			

7. guri 27J

9451	CCCCCCCCCC	CTGCTGCGCT GACGACGCGA		
9501		GGCTGGAAAG CCGACCTTTC		
9551	CCGGAGGGTT GGCCTCCCAA	ATTTTCCAAG TAAAAGGTTC		
9601		CGGACTGCGG GCCTGACGCC		
9651		GCAAATTCCT CGTTTAAGGA		
9701		GCATCCGGTG CGTAGGCCAC		
9751		AAGAGCAGCG TTCTCGTCGC		
9801		GGAGGGGCGA CCTCCCCGCT	 	
9851		CCCGCGCGCG		
9901		TGGCGCGGCT ACCGCGCCGA		
9951		AAGCGTGATA TTCGCACTAT		
10001		CCGCGAGGGA GGCGCTCCCT		
10051		GGCGCGAGCT CCGCGCTCGA		
10101	GCGCGAGGAG CGCGCTCCTC	GACTTTGAGC CTGAAACTCG		
10151	GCGCACACGT CGCGTGTGCA	ccecceccec		
10201	AACCAGGAGA TTGGTCCTCT	TTAACTTTCA AATTGAAAGT		
10251	TGTGGCGCGC ACACCGCGCG	GAGGAGGTGG CTCCTCCACC		
10301	TAAGCGCGCT ATTCGCGCGA	GGAGCAAAAC CCTCGTTTTG		

Figure 27K

				`	
10401			AGGGCCGCTG TCCCGGCGAC		
10451			CAGGAGCGCA GTCCTCGCGT		
10501			CATGCTTAGC GTACGAATCG		
10551			ACGTTCCCAT TGCAAGGGTA		
10601			GCGCTGAAGG CGCGACTTCC		
10651			GCGCATCCAC CGCGTAGGTG		
10701			GCGAGCTGAT CGCTCGACTA		
10751	TGGCTGGCAC ACCGACCGTG	GGGCAGCGGC CCCGTCGCCG	GATAGAGAGG CTATCTCTCC	CCGAGTCCTA GGCTCAGGAT	CTTTGACGCG GAAACTGCGC
10801			CCCAAGCCGA GGGTTCGGCT		
10851	GGCCGGACCT CCGGCCTGGA	GGGCTGGCGG CCCGACCGCC	TGGCACCCGC ACCGTGGGCG	GCGCGCTGGC CGCGCGACCG	AACGTCGGCG TTGCAGCCGC
10901			GACGATGAGT CTGCTACTCA		
10951			GATCAGATGA CTAGTCTACT		
11001	GCCACGCCC	CGGCGCTGCA GCCGCGACGT	GAGCCAGCCG CTCGGTCGGC	TCCGGCCTTA AGGCCGGAAT	ACTCCACGGA TGAGGTGCCT
11051	CGACTGGCGC GCTGACCGCG	CAGGTCATGG GTCCAGTACC	ACCGCATCAT TGGCGTAGTA	GTCGCTGACT CAGCGACTGA	GCGCGCAATC CGCGCGTTAG
11101	CTGACGCGTT GACTGCGCAA	CCGGCAGCAG GGCCGTCGTC	CCGCAGGCCA GGCGTCCGGT	ACCGGCTCTC TGGCCGAGAG	CGCAATTCTG GCGTTAAGAC
11151	GAAGCGGTGG CTTCGCCACC	TCCCGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC	CGCAAACCCC GCGTTTGGGG	ACGCACGAGA TGCGTGCTCT	AGGTGCTGGC TCCACGACCG
11201	GATCGTAAAC CTAGCATTTG	GCGCTGGCCGC	AAAACAGGGC TTTTGTCCCG	CATCCGGCCC GTAGGCCGGG	GACGAGGCCG CTGCTCCGGC
11251	GCCTGGTCTA CGGACCAGAT	CGACGCGCTG	CTTCAGCGCG GAAGTCGCGC	TGGCTCGTTA ACCGAGCAAT	CAACAGCGGC

Figure 27L

11351	GGCGCAGCGT CCGCGTCGCA	AGCAGCAGGG TCGTCGTCCC		
11401	••••	 ACACAGCCCG TGTGTCGGGC		
11451		 GAGCGCACTG CTCGCGTGAC		
11501		 AGTCTGGGCC TCAGACCCGG		
11551	•••••	 GTAAACCTGA CATTTGGACT		
11601		GGCTCCCACA CCGAGGGTGT		
13.651		CGCGCCTGTT GCGCGGACAA		
11701		TCCCGGGACA AGGGCCCTGT		
11751		 AGGTCAGGCG TCCAGTCCGC		
11801		 GCCGCGCGCT CGGCGCGCGA		
11851		 TACCTGCTGA ATGGACGACT		
11901		CAGCGAGGAG GTCGCTCCTC		
11951		 ACCTGATGCG TGGACTACGC		
12001	TGGCGCTGGA ACCGCGACCT	 CGCAACATGG GCGTTGTACC	-	
12051	AACCGGCCGT TTGGCCGGCA	CCTAATGGAC GGATTACCTG		
12101	CGTGAACCCC GCACTTGGGG	CCAATGCCAT GGTTACGGTA		
12151	CGCCCCTGG GCGGGGGACC	GGGGGATTCG CCCCCTAAGC		
12201	GGATTCCTCT CCTAAGGAGA	AGACGACAGC TCTGCTGTCG		

Figure 27 M

				•	
12301	AGGAAAGCTT	CCGCAGGCCA	AGCAGCTTGT	CCGATCTAGG	CGCTGCGGCC '
				GGCTAGATCC	
	1001110000	000010001	1001CG/MCh	oocingnice	oconcoccoo
12351				AGCTTGATAG	
	GGCGCCAGTC	TACGATCATC	GGGTAAAGGT	TCGAACTATC	CCAGAGAATG
12401	CAGCACTCGC	ACCACCCGCC	CGCGCCTGCT	GGGCGAGGAG	GAGTACCTAA
				CCCGCTCCTC	
	arcaranaca	100100000	ococooncur		CIUMIGONIII
			0100000111	****	m000000 mmm
12451				AAAACCTGCC	
	TGTTGAGCGA	CGACGTCGGC	GTCGCGCTTT	TTTTGGACGG	AGGCCGTAAA
12501	CCCAACAACG	GGATAGAGAG	CCTAGTGGAC	AAGATGAGTA	GATGGAAGAC
	GGGTTGTTGC	CCTATCTCTC	GGATCACCTG	TTCTACTCAT	CTACCTTCTG
12551	CTACCCCAC	GAGCACAGGG	ACCTCCCACC	CCCGCGCCCG	CCCACCCGTC
12331				GGGCGCGGGC	
	CATGCGCGTC	CICGIGICCC	1GCACGG1CC	GGGCGCGGGC	GOGIGGCAG
12601				TGTGGGAGGA	
	CAGTTTCCGT	GCTGGCAGTC	GCCCCAGACC	ACACCCTCCT	GCTACTGAGC
12651	GCAGACGACA	GCAGCGTCCT	GGATTTGGGA	GGGAGTGGCA	ACCCGTTTGC
	CGTCTGCTGT	CGTCGCAGGA	CCTAAACCCT	CCCTCACCGT	TGGGCAAACG
			••••	,	
12701	CCACCERTCCC	CCCACCCCCC	CCACAATCTT	TTAAAAAAAA	AAAAACCATC
12701					
	CGTGGAAGCG	GGGTCCGACC	CCTCTTACAA	TTTTTTTAA	TTTTTCGTAC
12751				GCACCGAGCG	
	TACGTTTTAT	TTTTTGAGTG	GTTCCGGTAC	CGTGGCTCGC	AACCAAAAGA
12801	TGTATTCCCC	TTAGTATGCG	GCGCGCGGCG	ATGTATGAGG	AAGGTCCTCC
				TACATACTCC	
	MCATTE 10000	1211011111000			
10051	managements of	01 01 0m0m00	max accessors	GCCAGTGGCG	CCCCCCCCCCC
12851					
	AGGGAGGATG	CTCTCACACC	ACTCGCGCCG	CGGTCACCGC	CGCCGCGACC
12901	GTTCTCCCTT	CGATGCTCCC	CTGGACCCGC	CGTTTGTGCC	TCCGCGGTAC
	CAAGAGGGAA	GCTACGAGGG	GACCTGGGCG	GCAAACACGG	AGGCGCCATG
12951	CTGCGGCCTA	CCGGGGGGAG	AAACAGCATC	CGTTACTCTG	AGTTGGCACC
				GCAATGAGAC	
	GACGCCGGAI	00000000	1110100170	00.2110.10.10	10.2.000100
		> = = > > > = = = = = = = = = = = = = =	moma como om	CCNCNACNAC	mc> > ccc> mc
13001	CCTATTCGAC				
	GGATAAGCTG	TGGTGGGCAC	ACATGGACCA	CCTGTTGTTC	AGTTGCCTAC
13051	TGGCATCCCT				
	ACCGTAGGGA	CTTGATGGTC	TTGCTGGTGT	CGTTGAAAGA	CTGGTGCCAG
13101	ATTCAAAACA	ATGACTACAG	CCCGGGGGGAG	GCAAGCACAC	AGACCATCAA
701U1				CGTTCGTGTG	
	IMAGITTIGT	INCIGNICIC	SSSCCCC IC	C011C01010	1C1GGINGII
					1 maamaa 1 5 1
13151					ATCCTGCATA
	AGAACTGCTG	GCCAGCGTGA	CCCCGCCGCT	GGACTTTTGG	TAGGACGTAT

Figure 27N

13251	CGGGTGATGG	TGTCGCGCTT	GCCTACTAAG	GACAATCAGG	TGGAGCTGAA
	GCCCACTACC	ACAGCGCGAA	CGGATGATTC	CTGTTAGTCC	ACCTCGACTT
					•
13301	ATACGAGTGG	GTGGAGTTCA	CGCTGCCCGA	GGGCAACTAC	TCCGAGACCA
	TATGCTCACC	CACCTCAAGT	GCGACGGGCT	CCCGTTGATG	AGGCTCTGGT
13351	TGACCATAGA	CCTTATGAAC	AACGCGATCG	TGGAGCACTA	CTTGAAAGTG
		GGAATACTTG			
13401	GGCAGACAGA	ACGGGGTTCT	GGAAAGCGAC	ATCGGGGTAA	AGTTTGACAC
		TGCCCCAAGA			
13451	CCGCAACTTC	AGACTGGGGT	TTGACCCCGT	CACTGGTCTT	GTCATGCCTG
		TCTGACCCCA			
13501	GGGTATATAC	AAACGAAGCC	TTCCATCCAG	ACATCATTTT	GCTGCCAGGA
		TTTGCTTCGG			
13551	TGCGGGGTGG	ACTTCACCCA	CAGCCGCCTG	AGCAACTTGT	TGGGCATCCG
••••		TGAAGTGGGT			
13601	CAAGCGGCAA	CCCTTCCAGG	AGGGCTTTAG	GATCACCTAC	GATGATCTGG
		GGGAAGGTCC			
13651	AGGGTGGTAA	CATTCCCGCA	CTGTTGGATG	TGGACGCCTA	CCAGGCGAGC
	TCCCACCATT	GTAAGGGCGT	GACAACCTAC	ACCTGCGGAT	GGTCCGCTCG
13701	TTGAAAGATG	ACACCGAACA	GGGCGGGGT	GGCGCAGGCG	GCAGCAACAG
	AACTTTCTAC	TGTGGCTTGT	CCCGCCCCCA	CCGCGTCCGC	CGTCGTTGTC
13751	CAGTGGCAGC	GGCGCGGAAG	AGAACTCCAA	CGCGGCAGCC	GCGGCAATGC
	GTCACCGTCG	CCGCGCCTTC	TCTTGAGGTT	GCGCCGTCGG	CGCCGTTACG
13801	AGCCGGTGGA	GGACATGAAC	GATCATGCCA	TTCGCGGCGA	CACCTTTGCC
	TCGGCCACCT	CCTGTACTTG	CTAGTACGGT	AAGCGCCGCT	GTGGAAACGG
13851		AGGAGAAGCG			
	TGTGCCCGAC	TCCTCTTCGC	GCGACTCCGG	CTTCGTCGCC	GGCTTCGACG
•		•			
13901	CGCCCCCGCT	GCGCAACCCG	AGGTCGAGAA	GCCTCAGAAG	AAACCGGTGA
	GCGGGGGCGA	CGCGTTGGGC	TCCAGCTCTT	CGGAGTCTTC	TTTGGCCACT
				•	
13951	TCAAACCCCT				
	AGTTTGGGGA	CTGTCTCCTG	TCGTTCTTTG	CGTCAATGTT	GGATTATTCG
					_
14001	AATGACAGCA				
	TTACTGTCGT	GGAAGTGGGT	CATGGCGTCG	ACCATGGAAC	GTATGTTGAT
14051	CGGCGACCCT				
	GCCGCTGGGA	GTCTGGCCTT	AGGCGAGTAC	CTGGGACGAA	ACGTGAGGAC
14101	ACGTAACCTG	CGGCTCGGAG	CAGGTCTACT	GGTCGTTGCC	AGACATGATG
	TGCATTGGAC	GCCGAGCCTC	GTCCAGATGA	CCAGCAACGG	TCTGTACTAC

Tigure 270

•				\	
14201		GAGCTGTTGC CTCGACAACG			
14251		CTCCCAACTC			
	TCCGGCAGAT	GAGGGTTGAG	TAGGCGGTCA	AATGGAGAGA	CTGGGTGCAC
14301		TTCCCGAGAA AAGGGCTCTT			
14351		GTCAGTGAAA CAGTCACTTT			
14401		CAACAGCATC GTTGTCGTAG			
14451		GCACCTGCCC CGTGGACGGG			
14501		CTATCGAGCC GATAGCTCGG			
14551		CAATAACACA GTTATTGTGT			
14601		CCAAGAAGCG GGTTCTTCGC			
14651	•	GCGCCCTGGG CGCGGGACCC			
14701		TGACGCCATC ACTGCGGTAG			
14751		CGCCACCAGT GCGGTGGTCA			
14801	*	GCCCGGCGCT CGGGCCGCGA			
14851	TAGCACGTCG ATCGTGCAGC	CCACCGCCGC GGTGGCGGCG			
14901	GCGGCCCTGC CGCCGGGACG	TTAACCGCGC AATTGGCGCG			
14951	GGCCGCTCGA CCGGCGAGCT	AGGCTGGCCG TCCGACCGGC			
15001	GGCGACGAGC CCGCTGCTCG	GGCCGCCGCA CCGGCGGCGT			
15051	GGTCGCAGGG CCAGCGTCCC	GCAACGTGTA CGTTGCACAT			

Figure 27P

15151	ACTTAGACTC	GTACTGTTGT	ATGTATCCAG	CGGCGGCGGC	GCGCAACGAA
10101				GCCGCCGCCG	
	TGAMICIGAG	CHIGHCHACK	INCNINGGIC	GCCGCCGCCG	CGCG11GC11
15201	GCTATGTCCA	AGCGCAAAAT	CAAAGAAGAG	ATGCTCCAGG	TCATCGCGCC
	CCATACAGGT	TCGCGTTTTA	GTTTCTTCTC	TACGAGGTCC	AGTAGCGCGG
	00				
				CC> CC> DD> C	*******
15251	GGAGATCTAT				
	CCTCTAGATA	CCGGGGGCT	TCTTCCTTCT	CGTCCTAATG	TTCGGGGCTT
15301	AGCTAAAGCG	GGTCAAAAAG	AAAAAGAAAG	ATGATGATGA	TGAACTTGAC
10001				TACTACTACT	
	TCGATTICGC	CCAGIIIIC	IIIIICIIIC	INCINCIACI	nc110mc10
15351				CCCAGGCGAC	
	CTGCTCCACC	TTGACGACGT	GCGATGGCGC	GGGTCCGCTG	CCCATGTCAC
	•				
15401	CANACCTCCA	CCCCTAAAAC	CTCTTTTCCC	ACCCGGCACC	ACCGTAGTCT
12401					
	CTTTCCAGCT	GCGCATTTTG	CACAAAACGC	TGGGCCGTGG	IGGCAICAGA
15451				ACAAGCGCGT	
	AATGCGGGCC	ACTCGCGAGG	TGGGCGTGGA	TGTTCGCGCA	CATACTACTC
15501	CTCTACCCC	ACCACCACCT	CCTTCACCAC	GCCAACGAGC	CCCTCGGGGA
13301				CGGTTGCTCG	
	CACATGCCGC	TGCTCCTGGA	CGAACTCGTC	CGGIIGCICG	CGGAGCCCC1
15551	GTTTGCCTAC	GGAAAGCGGC	ATAAGGACAT	GCTGGCGTTG	CCGCTGGACG
	CAAACGGATG	CCTTTCGCCG	TATTCCTGTA	CGACCGCAAC	GGCGACCTGC
25603	1000011000	*******	CTNNNCCCCC	TAACACTGCA	ĊC≱GGTGCTG
15601					
	TCCCGTTGGG	TTGTGGATCG	GATTTCGGGC	ATTGTGACGT	CGTCCACGAC
15651	CCCGCGCTTG	CACCGTCCGA	AGAAAAGCGC	GGCCTAAAGC	GCGAGTCTGG
	GGGCGCGAAC	GTGGCAGGCT	TCTTTTCGCG	CCGGATTTCG	CGCTCAGACC
		•			
15701	mc v cmmccc v	CCCACCCTCC	A COMO A TOOT	ACCCAAGCGC	CACCGACTGG
15/01					
	ACTGAACCGT	GGGTGGCACG	TCGACTACCA	TGGGTTCGCG	GIUGUIGACU
15751	AAGATGTCTT	GGAAAAAATG	ACCGTGGAAC	CTGGGCTGGA	GCCCGAGGTC
	TTCTACAGAA	CCTTTTTTAC	TGGCACCTTG	GACCCGACCT	CGGGCTCCAG
15001	CGCGTGCGGC	C3 3 MC3 3 CC3	CCTCCCCCC	CCACTCCCCC	тсерересст
12801	Cecerecee	CAATCAAGCA	GGIGGCGCCG	GGAC1GGGCG	1007070001
	GCGCACGCCG	GTTAGTTCGT	CCACCGCGGC	CCTGACCCGC	ACGICIGGCA
15851	GGACGTTCAG	ATACCCACTA	CCAGTAGCAC	CAGTATTGCC	ACCGCCACAG
				GTCATAACGG	
4.6000	AGGGCATGGA	010101110	mana a comma	CCTCACCCC	CCCCCATCCC
15901	AGGGCATGGA	GACACAAACG	100000116	CCICAGCGGI	GGCGGWIGCC
	TCCCGTACCT	CTGTGTTTGC	AGGGGCCAAC	GGAGTCGCCA	CCGCCTACGG
15951	GCGGTGCAGG	CGGTCGCTGC	GGCCGCGTCC	AAGACCTCTA	CGGAGGTGCA
	CCCCACCACC	CCCACCCACC	CCGCCGCTGC	TTCTGGAGAT	GCCTCCACGT
	COCCUCATO	SCURGEGREG	-caccacuad		
				000000000	COCCCCCC
16001	AACGGACCCG				
	TTGCCTGGGC	ACCTACAAAG	CGCAAAGTCG	GGGGGCCGCG	GGCGCGGCAA

Figure 270

WO 02/022080 PCT/US01/28861

16051	CCACCACTA	recerecee	אככככככתאכ	TGCCCGAATA	CCCCTACAT
10031				ACGGGCTTAT	
	GCTCCTTCAT	GCCGCGGCGG	ICGCGCGAIG	ACGGGCTTAT	ACGGGAIGIA
	000000000000	00000000000	000000000000	00000000	1000000000
16101				GGCTACACCT	
	GGAAGGTAAC	GCGGATGGGG	GCCGATAGCA	CCGATGTGGA	TGGCGGGTC
16151				CACTGGAACC	
	TTCTGCTCGT	TGATGGGCTG	CGGCTTGGTG	GTGACCTTGG	GCGGCGGCGG
16201	GTCGCCGTCG	CCAGCCCGTG	CTGGCCCCGA	TTTCCGTGCG	CAGGGTGGCT
	CAGCGGCAGC	GGTCGGGCAC	GACCGGGGCT	AAAGGCACGC	GTCCCACCGA
16251	CGCGAAGGAG	GCAGGACCCT	GGTGCTGCCA	ACAGCGCGCT	ACCACCCCAG
	GCGCTTCCTC	CGTCCTGGGA	CCACGACGGT	TGTCGCGCGA	TGGTGGGGTC
16301	CATCGTTTAA	AAGCCGGTCT	TTGTGGTTCT	TGCAGATATG	GCCCTCACCT
	GTAGCAAATT	TTCGGCCAGA	AACACCAAGA	ACGTCTATAC	CGGGAGTGGA
					•
16351	GCCGCCTCCG	TTTCCCGGTG	CCGGGATTCC	GAGGAAGAAT	GCACCGTAGG
				CTCCTTCTTA	
16401	AGGGGCATGG	CCGGCCACGG	CCTGACGGGC	GGCATGCGTC	GTGCGCACCA
20102				CCGTACGCAG	
16451	CCGCCGCGG	CGCGCGTCGC	ACCGTCGCAT	GCGCGGCGGT	ATCCTGCCCC
10431				CGCGCCGCCA	
	GGCCGCCGCC	GCGCGCAGCG	1000		
16501	ጥር ርጥጥ አጥጥር ር	እርተር <b>እ</b> ቸርርርር	GCGGCGATTG	GCGCCGTGCC	CGGAATTGCA
10201		and the second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second second s		CGCGGCACGG	
	AGGAATAAGG	IGACIAGOGG	COCCOCIANC	66666677666	00011121001
16551	maccamacca com	mccacccca	CACACACTCA	TTAAAAACAA	CTTCC ATCTC
10001				AATTTTTGTT	
	AGGCACCGGA	¥CG1CCGCG1	CICIGIGACI	AATTTTTGTT	CAACGIACAC
16601	0111110011	******	CDCC X CDCDC	ACGCTCGCTT	CCTCCTCTAA
16601					
	CTTTTTAGTT	TTATTTTCA	GACCIGAGAG	TGCGAGCGAA	CCAGGACATT
			G1.0011.0000	COCMCMCMCC	CCCCCCACA
16651				GCGTCTCTGG	
	GATAAAACAT	CTTACCTTCT	GTAGTTGAAA	CGCAGAGACC	GGGGGGGTGT
					1001001101
16701	CGGCTCGCGC				
	GCCGAGCGCG	GGCAAGTACC	CTTTGACCGT	TCTATAGCCG	TGGTCGTTAT
16751	TGAGCGGTGG	CGCCTTCAGC	TGGGGCTCGC	TGTGGAGCGG	CATTAAAAAT
	ACTCGCCACC	GCGGAAGTCG	ACCCCGAGCG	ACACCTCGCC	GTAATTTTTA
16801	TTCGGTTCCA				
	AAGCCAAGGT	GGCAATTCTT	GATACCGTCG	TTCCGGACCT	TGTCGTCGTG
16851					CAACAAAAGG
	TCCGGTCTAC	GACTCCCTAT	TCAACTTTCT	CGTTTTAAAG	GTTGTTTTCC
16901					CCTGGCCAAC
	ACCATCTACC	GGACCGGAGA	CCGTAATCGC	CCCACCACCT	GGACCGGTTG
16951	CAGGCAGTGC	AAAATAAGAT	TAACAGTAAG	CTTGATCCCC	GCCCTCCCGT
					CGGGAGGGCA

Figure 27R

17051	AAAAGCGTCC	GCGCCCCGAC	AGGGAAGAAA	CTCTGGTGAC	GCAAATAGAC
	TTTTCGCAGG	CGCGGGGCTG	TCCCTTCTTT	GAGACCACTG	CGTTTATCTG
17161	GAGCCTCCCT	CGTACGAGGA	GGCACTAAAG	CAAGGCCTGC	CCACCACCCG
	CTCGGAGGGA	GCATGCTCCT	CCGTGATTTC	GTTCCGGACG	GGTGGTGGGC
17151	TCCCATCGCG	CCCATGGCTA	CCGGAGTGCT	GGGCCAGCAC	ACACCCGTAA
_,		GGGTACCGAT	<del>-</del>		
17201	CGCTGGACCT	GCCTCCCCC	GCCGACACCC	AGCAGAAACC	TGTGCTGCCA
2,202		CGGAGGGGG		-	
	400001				
17251	GGCCCGACCG	CCGTTGTTGT	AACCCGTCCT	AGCCGCGCGT	CCCTGCGCCG
		GGCAACAACA			
17301	CGCCGCCAGC	GGTCCGCGAT	CGTTGCGGCC	CGTAGCCAGT	GGCAACTGGC
•		CCAGGCGCTA			
17351	AAAGCACACT	GAACAGCATC	GTGGGTCTGG	GGGTGCAATC	CCTGAAGCGC
	TTTCGTGTGA	CTTGTCGTAG	CACCCAGACC	CCCACGTTAG	GGACTTCGCG
17401	CGACGATGCT	TCTGATAGCT	AACGTGTCGT	ATGTGTGTCA	TGTATGCGTC
	GCTGCTACGA	AGACTATCGA	TTGCACAGCA	TACACACAGT	ACATACGCAG
		•			
17451	CATGTCGCCG	CCAGAGGAGC	TGCTGAGCCG	CCGCGCGCCC	GCTTTCCAAG
	GTACAGCGGC	GGTCTCCTCG	ACGACTCGGC	GGCGCGCGG	CGAAAGGTTC
17501		CTTCGATGAT			
	TACCGATGGG	GAAGCTACTA	CGGCGTCACC	AGAATGTACG	TGTAGAGCCC
17551		TCGGAGTACC			
	GGTCCTGCGG	AGCCTCATGG	ACTCGGGGCC	CGACCACGTC	AAACGGGCGC
17601	CCACCGAGAC	GTACTTCAGC	CTGAATAACA	AGTTTAGAAA	CCCCACGGTG
	GGTGGCTCTG	CATGAAGTCG	GACTTATTGT	TCAAATCTTT	GGGGTGCCAC
17651		ACGACGTGAC			
	CGCGGATGCG	TGCTGCACTG	GTGTCTGGCC	AGGGTCGCAA	ACTGCGACGC
		•			
17701	GTTCATCCCT				
	CAAGTAGGGA	CACCTGGCAC	TCCTATGACG	CATGAGCATG	TTCCGCGCCA
17751	TCACCCTAGC				
	AGTGGGATCG	ACACCCACTA	TTGGCACACG	ACCTGTACCG	AAGGTGCATG
17801	TTTGACATCC				
	AAACTGTAGG	CGCCGCACGA	CCTGTCCCCG	GGATGAAAAT	TCGGGATGAG
				:	
17851	TGGCACTGCC				
	ACCGTGACGG	ATGTTGCGGG	ACCGAGGGTT	CCCACGGGGT	TTAGGAACGC
17901	AATGGGATGA				
	TTACCCTACT	TCGACGATGA	CGAGAACTTT	ATTTGGATCT	TCTTCTCCTG

Figure 275

WO 02/0	22080	<b>\</b>			PCT/US01/28861
17951	GATGACAA	AAGACGAAGT	AGACGAGCAA	GCTGAGCAGC	AA ACTCA
	-		TCTGCTCGTT		_
18001	CGTATTTGGG	CAGGCGCCTT	ATTCTGGTAT	AAATATTACA	AAGGAGGGTA
	GCATAAACCC	GTCCGCGGAA	TAAGACCATA	TTTATAATGT	TTCCTCCCAT
18051	TTCAAATAGG				
					ATTTTGTAAA
18101	CAACCTGAAC				
			TCTTAGAGTC		
18151	TCATGCAGCT				
					TTTGGTACAA
18201	ACGGTTCATA				TCCGTAAGAA
18251	GTAAAGCAAC				ACGTTAAAAA
	CATTICGITG	IIIIACCIII	CGAICITICA	GIICACCIII	ACO1112221
18301	CTCAACTACT	GAGGCAGCCG	CAGGCAATGG	TGATAACTTG	ACTCCTAAAG
			GTCCGTTACC		
18351	TGGTATTGTA				
					GTGAGTATAA
18401	TCTTACATGC				
					ATTACCCGGT
18451	ACAATCTATG				
					CTGTTAAAAT
18501	TTGGTCTAAT				AGACCGCCCG
18551					GAAACACAGA
18221					CTTTGTGTCT
10601	GCTTTCATAC				
18001	CGAAAGTATG	CACCITITIC	AACTAAGGTA	ACCACTATCT	TGGTCCATGA
	CGAAAGIAIG	GICGABBBCO			
18651	TTTCTATGTG				
					ACAATCTTAA
18701	ATTGAAAATC	ATGGAACTGA	AGATGAACTT	CCAAATTACT	GCTTTCCACT
					CGAAAGGTGA
18751	GGGAGGTGTG	ATTAATACAG	AGACTCTTAC	CAAGGTAAAA	CCTAAAACAG
					GGATTTTGTC
18801	GTCAGGAAAA				
•					TCTATTTTA
18851	GAAATAAGAG				
	CTTTATTCTC	AACCTTTATT	AAAACGGTAC	CTTTAGTTAG	ATTTACGGTT

Figure 27T

18951		AACGTAAAAA TTGCATTTTT	
19001		AGTGGTGGCT TCACCACCGA	
19051		GGTCCCTTGA CCAGGGAACT	
19101	 	GCTGGCCTGC CGACCGGACG	
19151		CTTCCACATC GAAGGTGTAG	
19201		TCCTGCCGGG AGGACGGCCC	
19251		ATGGTTCTGC TACCAAGACG	
19301		CATTAAGTTT GTAATTCAAA	
19351		ACAACACCGC TGTTGTGGCG	
19401		CAGTCCTTTA GTCAGGAAAT	
19451		CGCCAACGCT GCGGTTGCGA	
19501		CTTTCCGCGG GAAAGGCGCC	
19551		CTGGGCTCGG GACCCGAGCC	
19601		CCTAGATGGA GGATCTACCT	
19651		CCTTTGACTC GGAAACTGAG	TGGCCTGGCA ACCGGACCGT
19701		AACGAGTTTG TTGCTCAAAC	
19751			ACTGGTTCCT TGACCAAGGA
19801			TTCTATATCC AAGATATAGG

Figure 27 4

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19851	CAGAGAGCTA	CAAGGACCGC	ATGTACTCCT	TCTTTAGAAA	TCCAGCCC
	GTCTCTCGAT	GTTCCTGGCG	TACATGAGGA	AGAAATCTTT	GAAGGTCGGG
19901	ATGAGCCGTC	AGGTGGTGGA	TGATACTAAA	TACAAGGACT	ACCAACAGGT
	TACTCGGCAG	TCCACCACCT	ACTATGATTT	ATGTTCCTGA	TGGTTGTCCA
19951	GGGCATCCTA	CACCAACACA	ACAACTCTGG	ATTTGTTGGC	TACCTTGCCC
	CCCGTAGGAT	GTGGTTGTGT	TGTTGAGACC	TAÄACAACCG	ATGGAACGGG
20001	CCACCATGCG	CGAAGGACAG	GCCTACCCTG	CTAACTTCCC	CTATCCGCTT
	GGTGGTACGC	GCTTCCTGTC	CGGATGGGAC	GATTGAAGGG	GATAGGCGAA
20051	ATAGGCAAGA	CCGCAGTTGA	CAGCATTACC	CAGAAAAAGT	TTCTTTGCGA
	TATCCGTTCT	GGCGTCAACT	GTCGTAATGG	GTCTTTTTCA	AAGAAACGCT
20101		TGGCGCATCC	*		
		ACCGCGTAGG			
20151		CCTGGGCCAA			
		GGACCCGGTT			
20201	. • • • • • • • • • • • • • • • • • • •	CTTTTGAGGT			
		GAAAACTCCA			
20251		GAAGTCTTTG			
		CTTCAGAAAC			
20301		AACCGTGTAC			
		TTGGCACATG			
20351		GAAGCAAGCA			
		CTTCGTTCGT			
20401		AACTGAAAGC			
		TTGACTTTCG			
20451		ACCTATGACA			
		TGGATACTGT			
20501	AGCTCGCCTG				
		GCGGTATCAG			
20551					GCTACCTCTT
					CGATGGAGAA
20601	TGAGCCCTTT ACTCGGGAAA	GGCTTTTCTG CCGAAAAGAC			
					0000030000
20651	AGTACGAGTC				
		TGAGGACGCG			
20701	TGTATAACGC				
		ACCTTTTCAG			
20751					GCCAACTGGC
	GCGGACACCT	GATAAGACGA	CGTACAAAGA	GGTGCGGAAA	CGGTTGACCG

Figure 27 V.

20851		TGCTCAACAG ACGAGTTGTC			
20901		CTCTACAGCT			
	GGTCCTTGTC	GAGATGTCGA	AGGACCTCGC	GGTGAGCGGG	ATGAAGGCGT
20951	GCCACAGTGC	GCAGATTAGG	AGCGCCACTT	CTTTTTGTCA	CTTGAAAAAC
	CGGTGTCACG	CGTCTAATCC	TCGCGGTGAA	GAAAAACAGT	GAACTTTTTG
21001	ATCTAAAAAT	AATGTACTAG	AGACACTTTC	AATAAAGGCA	AATGCTTTTA
21001		TTACATGATC			
21051		CTCGGGTGAT			
	AAACATGTGA	GAGCCCACTA	ATAAATGGGG	GTGGGAACGG	CAGACGCGGC
21101	ተተተልልልልልጥር	AAAGGGGTTC	TGCCGCGCAT	CGCTATGCGC	CACTGGCAGG
21101	באדידידיאה	TTTCCCCAAG	ACGGCGCGTA	GCGATACGCG	GTGACCGTCC
21151		GATACTGGTG			
	CTGTGCAACG	CTATGACCAC	AAATCACGAG	GTGAATTTGA	GTCCGTGTTG
					0001001001
21201	CATCCGCGGC	AGCTCGGTGA	AGTTTTCACT	CCACAGGCTG	CGCACCATCA
	GTAGGCGCCG	TCGAGCCACT	TCAAAAGTGA	GGTGTCCGAC	GCGTGGTAGT
21251	CCAACGCGTT	TAGCAGGTCG	GGCGCCGATA	TCTTGAAGTC	GCAGTTGGGG
21231	GGTTGCGCAA	ATCGTCCAGC	CCGCGGCTAT	AGAACTTCAG	CGTCAACCCC
	0011000				
21301	CCTCCGCCCT	GCGCGCGCGA	GTTGCGATAC	ACAGGGTTGC	AGCACTGGAA
	GGAGGCGGGA	CGCGCGCGCT	CAACGCTATG	TGTCCCAACG	TCGTGACCTT
21351	CACTATCAGC	GCCGGGTGGT	GCACGCTGGC	CAGCACGCTC	TTGTCGGAGA
	GTGATAGTCG	CGGCCCACCA	CGTGCGACCG	GTCGTGCGAG	AACAGCCTCT
21401	TONGATOGO	GTCCAGGTCC	TCCGCGTTGC	TCAGGGCGAA	CGGAGTCAAC
21901	AGTCTAGGCG	CAGGTCCAGG	AGGCGCAACG	AGTCCCGCTT	GCCTCAGTTG
	Adicinocc	0.,001.00.00			
21451	TTTGGTAGCT	GCCTTCCCAA	AAAGGGCGCG	TGCCCAGGCT	TTGAGTTGCA
	AAACCATCGA	CGGAAGGGTT	TTTCCCGCGC	ACGGGTCCGA	AACTCAACGT
			•		_
21501	CTCGCACCGT	AGTGGCATCA	AAAGGTGACC	GTGCCCGGTC	TGGGCGTTAG
	GAGCGTGGCA	TCACCGTAGT	TTTCCACTGG	CACGGGCCAG	ACCCGCAATC
21551	GATACAGCGC	СТССАТААА	GCCTTGATCT	GCTTAAAAGC	CACCTGAGCC
21551	CHARCACCC	CACCTATTT	CGCAACTAGA	CGAATTTTCG	GTGGACTCGG
	CINIGICACA				
21601	TTTGCGCCTT	CAGAGAAGAA	CATGCCGCAA	GACTTGCCGG	AAAACTGATT
	AAACGCGGAA	GTCTCTTCTT	GTACGGCGTT	CTGAACGGCC	TTTTGACTAA
				,	
21651	GGCCGGACAG	GCCGCGTCGT	GCACGCAGCA	CCTTGCGTCG	GTGTTGGAGA
	CCGGCCTGTC	CGGCGCAGCA	CGTGCGTCGT	GGAACGCAGC	CACAACCTCT
			CACCCMMCM	<b>- ԻՐ ՖՐԸ Ֆ</b> ԴՐ ԻՐ	GGCCTTGCTA
21701	TCTGCACCAC	. Manage CCCCCC	CACCCCCAACA	VCACCATCII	CCGGAACGAT
	ACACC TGG TG	**************************************	argaceway.		

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•					
21801					CACTTAAGCT GTGAATTCGA
21851		CTCAGCGCAG			
	GCGGAAGCTA	GAGTCGCGTC	GCCACGTCGG	TGTTGCGCGT	CGGGCACCCG
21901		TGTAGGTCAC ACATCCAGTG			
21951		ATCATCGTCA TAGTAGCAGT			
22001	GCAACCCGCG	GTGCTCCTCG	TTCAGCCAGG	TCTTGCATAC	GGCCGCCAGA
•	CGTTGGGCGC	CACGAGGAGC	AAGTCGGTCC	AGAACGTATG	CCGGCGGTCT
22051		GGTCAGGCAG CCAGTCCGTC			
22101		TTGTCCATCA AACAGGTAGT			
22151		GATCGGCACA			
22151		CTAGCCGTGT			
22201		TGGGCTCTTC ACCCGAGAAG			
22251		TCTTCATTCA AGAAGTAAGT			
2222					
22301		TAGCACCGGT ATCGTGGCCA			
22351		TTTCTTCCTC AAAGAAGGAG			
22401		TTGGGAGAAG AACCCTCTTC			
22451	CCAAATCCGC	CGCCGAGGTC GCGGCTCCAG			
22501	AGCGCGTCTT TCGCGCAGAA	CACTACTCAG			
22551	CATCCGCTTT GTAGGCGAAA	TTTGGGGGCG AAACCCCCGC			
22601	ACGACACGTC TGCTGTGCAG				GCGTCCGCGC CGCAGGCGCG
22651	TCGGGGGTGG AGCCCCCACC	TTTCGCGCTG AAAGCGCGAC			

Figure 27 X

22751			 CCACCGATGC GGTGGCTACG	
22801			CTTGAGGAGG GAACTCCTCC	
22851			 AGACGACGAG TCTGCTGCTC	
22901			 ACAACGCAGA TGTTGCGTCT	
22951			 GGCGACTACC CCGCTGATGG	
23001			CCAGTGCGCC GGTCACGCGG	
23051			TCGCCATAGC AGCGGTATCG	
23101		-	CGCGTACCCC GCGCATGGGG	
23151			CCTCAACTTC GGAGTTGAAG	
23201			ACATCTTTTT TGTAGAAAAA	
23251			AGCCGAGCGG TCGGCTCGCC	
23301			TATCGCCTCG ATAGCGGAGC	
23351			ACGAGAAGCG TGCTCTTCGC	
23401	GCTCTGCAAC CGAGACGTTG		AGTCACTCTG TCAGTGAGAC	
23451	GGAACTCGAG CCTTGAGCTC		CGTACTAAAA GCATGATTTT	
23501	AGGTCACCCA TCCAGTGGGT		ACCTACCCC TGGATGGGGG	
23551	AGCACAGTCA TCGTGTCAGT		CGTGCGCAGC GCACGCGTCG	
23601	GGATGCAAAT CCTACGTTTA		GGGCCTACCC CCCGGATGGG	

Figure 27 Y

	-				
23701	GAGCGACGCA	AACTAATGAT	GGCCGCAGTG	CTCGTTACCG	TGGAGCTTGA
			CCGGCGTCAC		
23751			CTGACCCGGA		
	CACGTACGTC	GCCAAGAAAC	GACTGGGCCT	CTACGTCGCG	TTCGATCTCC
23801	AAACATTGCA	CTACACCTTT	CGACAGGGCT	ACGTACGCCA	GGCCTGCAAG
	TTTGTAACGT	GATGTGGAAA	GCTGTCCCGA	TGCATGCGGT	CCGGACGTTC
23851	ATCTCCAACG	TGGAGCTCTG	CAACCTGGTC	TCCTACCTTG	GAATTTTGCA
			GTTGGACCAG		
23901			ACGTGCTTCA		
			TGCACGAAGT		
23951			GACTGCGTTT		
			CTGACGCAAA		
24001			TTGGCAGCAG		
			AACCGTCGTC		
24051			TAAAGCAAAA		
			ATTTCGTTTT		
24101			GCCGCGCACC		
			CGGCGCGTGG		
24151			GCAACAGGGT		
			CGTTGTCCCA		
24201			GGAACTTTAT		
			CCTTGAAATA		
24251			CTTCCTAGCG		
			GAAGGATCGC		
24301			TTGGGGCCAC		
			AACCCCGGTG		
24351	CAACTACCTT	GCCTACCACT	CTGACATAAT	GGAAGACGTG	AGCGGTGACG
					TCGCCACTGC
24401	GTCTACTGGA	GTGTCACTGT	CGCTGCAACC	TATGCACCCC	GCACCGCTCC
	•				CGTGGCGAGG
24451	CTGGTTTGCA	ATTCGCAGCT	GCTTAACGAA	AGTCAAATTA	TCGGTACCTT
					AGCCATGGAA
24501	TGAGCTGCAG	GGTCCCTCGC	CTGACGAAAA	GTCCGCGGCT	CCGGGGTTGA
					GGCCCCAACT
24551	AACTCACTCC	GGGGCTGTGG	ACGTCGGCTT	ACCTTCGCAA	ATTTGTACCT
	TTGAGTGAGG	CCCCGACACC	TGCAGCCGAA	TGGAAGCGTT	TAAACATGGA

Figure 27Z

WO 02/022	2080				PCT/US01/28861
24601	GAGGACC	ACGCCCACGA	GATTAGGTTC	TACGAAGACC	CCCCCC
• • • • • • • • • • • • • • • • • • • •				ATGCTTCTGG	
24651	GCCTAATGCG	GAGCTTACCG	CCTGCGTCAT	TACCCAGGGC	CACATTCTTG
	CGGATTACGC	CTCGAATGGC	GGACGCAGTA	ATGGGTCCCG	GTGTAAGAAC
24701	GCCAATTGCA	AGCCATCAAC	AAAGCCCGCC	<b>AAGAGTTTCT</b>	GCTACGAAAG
	CGGTTAACGT	TCGGTAGTTG	TTTCGGGCGG	TTCTCAAAGA	CGATGCTTTC
24751	GGACGGGGGG				
				CCGCTCCTCG	
24801				GCCGCGGGCC	
0.4053				CGGCGCCCGG	
24851	AGGATGGCAC			+	
24001				GGCGGCGGTG	
24901	GGAGGAATAC			CCAAAACCTG	
24951	AGGACATGAT				
24701				TGCTCCTTCG	
25001	GAAGAGGTGT	CAGACGAAAC	ACCGTCACCC	TCGGTCGCAT	TCCCCTCGCC
	CTTCTCCACA	GTCTGCTTTG	TGGCAGTGGG	AGCCAGCGTA	AGGGGAGCGG
25051	GGCGCCCCAG	AAATCGGCAA	CCGGTTCCAG	CATGGCTACA	ACCTCCGCTC
	CCGCGGGGTC	TTTAGCCGTT	GGCCAAGGTC	GTACCGATGT	TGGAGGCGAG
25101	CTCAGGCGCC	GCCGGCACTG	CCCGTTCGCC	GACCCAACCG	TAGATGGGAC
	GAGTCCGCGG	CGGCCGTGAC	GGGCAAGCGG	CTGGGTTGGC	ATCTACCCTG
25151	ACCACTGGAA				
0.5001			•	GTCGGCGCCG	
25201	AGAGCAACAA				
25251	CCATAGTTGC			TACCGCGCCC	
25251				CGTTGTAGAG	
25201	CGCTTTCTTC				
25501				AAGGGGGCAT	
25351	TTACTACCGT	CATCTCTACA	GCCCATACTG	CACCGGCGGC	AGCGGCAGCA
20001				GTGGCCGCCG	
25401	ACAGCAGCGG	CCACACAGAA	GCAAAGGCGA	CCGGATAGCA	AGACTCTGAC
				GGCCTATCGT	
25451	AAAGCCCAAG	AAATCCACAG	CGGCGGCAGC	AGCAGGAGGA	GGAGCGCTGC
				TCGTCCTCCT	
25501	GTCTGGCGCC				
	CAGACCGCGG	GTTGCTTGGG	CATAGCTGGG	${\tt CGCTCGAATC}$	TTTGTCCTAA

Figure 27 AA

WO 02/0220	080				PCT/US01/28	861
25551	TTTCC	TGTATGCTAT	ATTTCAACAG	AGCAGGGGCC	AACAAGA	
				TCGTCCCCGG		
25601	GCTGAAAATA	AAAAACAGGT	CTCTGCGATC	CCTCACCCGC	AGCTGCCTGT	
				GGAGTGGGCG		
25651	ATCACAAAAG					
				GCGACCTTCT		
25701	CTCTTCAGTA					
	•			TTCCTGATCA		
25751				TCTCCAGCGG		
				AGAGGTCGCC		
25801	CGCCAGCACC					
				CGTTCCTTTA		
25851				CTTGCGGCTG GAACGCCGAC		
	ATGTACACCT	CAAIGGICGG	IGITIACCCI	GAACGCCGAC	CICOACOOOI	
25901	AGACTACTCA	ACCCGAATAA	ACTACATGAG	CGCGGGACCC	CACATGATAT	
	TCTGATGAGT	TGGGCTTATT	TGATGTACTC	GCGCCCTGGG	GTGTACTATA	
25951	CCCGGGTCAA					
				TGGCTTAAGA		
26001	GCGGCTATTA					
				GAATTAGGGG		
26051				TCCCACCACT		
				AGGGTGGTGA		
26101	CCAGAGACGC			GATTGAGTCC		
26151				CCCGGGCAGG		
26151				GGGCCCGTCC		
26201	CCTGACAATC					
20201	CCIGACAAIC	TCTCCCGCTC	CATAAGTCGA	GTTGCTGCTC	AGCCACTCGA	
26251	CCTCGCTTGG	TCTCCGTCCG	GACGGGACAT	TTCAGATCGG	CGGCGCCGGC	
				AAGTCTAGCC		
26301	CGCTCTTCAT	TCACGCCTCG	TCAGGCAATC	CTAACTCTGC	AGACCTCGTC	
				GATTGAGACG		
26351	CTCTGAGCCG	CGCTCTGGAG	GCATTGGAAC	TCTGCAATTT	ATTGAGGAGT	
	٠			AGACGTTAAA		
26401	TTGTGCCATC					
_0.2	•			GCCCTGGAGG	•	
26451	CCGGATCAAT					
	GGCCTAGTTA	AATAAGGATT	GAAACTGCGC	CATTTCCTGA	GCCGCC10CC	

Figure 27 AB

WO 02/022080 PCT/US01/28861										
26501		ATGTTAAGTG TACAATTCAC								
26551	TGGTCCACTG ACCAGGTGAC	TCGCCGCCAC AGCGGCGGTG	AAGTGCTTTG TTCACGAAAC	CCCGCGACTC GGGCGCTGAG	CGGTGAGTTT GCCACTCAAA					
26601	TGCTACTTTG ACGATGAAAC	AATTGCCCGA TTAACGGGCT								
26651	CCGGCTTACC GGCCGAATGG	GCCCAGGGAG CGGGTCCCTC								
26701		CCTGCTAGTT GGACGATCAA								
26751	GTGATTTGCA CACTAAACGT	ACTGTCCTAA TGACAGGATT								
26801	TCTCTGTGCT AGAGACACGA	GAGTATAATA CTCATATTAT								
26851	TATCGCCATC ATAGCGGTAG	CTGTAAACGC GACATTTGCG								
26901	GCGAACCTTA CGCTTGGAAT	CCTGGTACTT GGACCATGAA								
26951	GTTTCAACCC CAAAGTTGGG	AGACGGAGTG TCTGCCTCAC								
27001	TACTCCATCA ATGAGGTAGT	GAAAAAACAC CTTTTTTGTG								
27051	TGCGTCACCG ACGCAGTGGC	GCCGCTGCAC CGGCGACGTG	CACACCTACC GTGTGGATGG	GCCTGACCGT CGGACTGGCA	AAACCAGACT TTTGGTCTGA					
27101	TTTTCCGGAC AAAAGGCCTG	AGACCTCAAT TCTGGAGTTA	AACTCTGTTT TTGAGACAAA	ACCAGAACAG TGGTCTTGTC	GAGGTGAGCT CTCCACTCGA					
27151	TAGAAAACCC ATCTTTTGGG	TTAGGGTATT AATCCCATAA								
27201	TGAACAATTC ACTTGTTAAG	AAGCAACTCT TTCGTTGAGA								
27251	ATCGGGGTTG TAGCCCCAAC	GGGTTATTCT CCCAATAAGA								
27301	AACGCTTCTC TTGCGAAGAG	TGCCTAAGGC ACGGATTCCG	TCGCCGCCTG AGCGGCGGAC	CTGTGTGCAC GACACACGTG	ATTTGCATTT TAAACGTAAA					
27351	ATTGTCAGCT		TGGGGTCGCC	ACCCAAGATG	ATTAGGTACA					
27401	TAATCCTAGG ATTAGGATCC	TTTACTCACC	CTTGCGTCAG GAACGCAGTC	CCCACGGTAC GGGTGCCATG	CACCCAAAAG GTGGGTTTTC					

Figure 27AC

O 02/022	080				PCT/US01/28861
27451	GTGGA'	AGGAGCCAGC	CTGTAATGTT	ACATTCGCAG	
	CACCTAAAAT	TCCTCGGTCG	GACATTACAA	TGTAAGCGTC	GACTTCGATT
				AGAACATGAA	
•	ACTCACGTGG	TGAGAATATT	TTACGTGGTG	TCTTGTACTT	TTCGACGAAT
27551				CTGTTTATGC	
	AAGCGGTGTT	ŢTTGTTTTAA	CCGTTCATAC	GACAAATACG	ATAAACCGTC
27601				GTTTTCCAGG	
				CAAAAGGTCC	
27651				TGAAATGTGC	
				ACTTTACACG	
27701				CCCCACAAAA	
				GGGGTGTTTT	
27751				CTAATTACAG	
				GATTAATGTC	
27801				AAGCAGACGC	
				TTCGTCTGCG	
27851				TACAAAGCTA	
				ATGTTTCGAT	
27901				ATTCAAAAAG	
				TAAGTTTTTC	
27951				TTTCCTGCTC	
				AAAGGACGAG	
28001				CTCCAGCGCT GAGGTCGCGA	
28051				TTTGGCCAGC AAACCGGTCG	
				•	
28101				GTGGGATTGT	GAGATGACCA
28151				TTACATCTAC AATGTAGATG	
28201				GATAACTTGG	
				CTATTGAACC	•
28251				TATTATTATG	
				ATAATAATAC	
28301					TCCCATCATT
					AGGGTAGTAA
28351					GACTGAAACA
	CACGATGTGG	GTTTGTTACT	ACCTTAGGTA	TCTAACCTGC	CTGACTTTGT

Figure 27AD

28451	TTTTATATTA	CTGACCCTTG	TTGCGCTTTT	TTGTGCGTGC	TCCACATTGG
20431				AACACGCACG	
	WWWINIWWI	GAC I GGGAAC	ANCUCGAMA	ANCHOUNCE	MOGIGIANCC
28501				TTCCAGCCTT	
	GACGCCAAAG	AGTGTAGCTT	CATCTGACGT	AAGGTCGGAA	GTGTCAGATA
28551	TTGCTTTACG	GATTTGTCAC	CCTCACGCTC	ATCTGCAGCC	TCATCACTGT
	AACGAAATGC	CTAAACAGTG	GGAGTGCGAG	TAGACGTCGG	AGTAGTGACA
28601		መጥጥ አጥር ር አርጥ	CCAMMCACMC	GGTCTGTGTG	רכריייייכראיי
20001				=	
	CCAGTAGCGG	AAATAGGTCA	CGTAACTGAC	CCAGACACAC	GCGAAACGIA
	•				
28651	ATCTCAGACA	CCATCCCCAG	TACAGGGACA	GGACTATAGC	TGAGCTTCTT
	TAGAGTCTGT	GGTAGGGGTC	ATGTCCCTGT	CCTGATATCG	ACTCGAAGAA
28701	AGAATTCTTT	AATTATGAAA	TTTACTGTGA	CTTTTCTGCT	GATTATTTGC
	TCTTAAGAAA	TTAATACTTT	AAATGACACT	GAAAAGACGA	CTAATAAACG
28751	<b>እ</b> ርርርጥ <u>እጥር</u> ጥር	ССФФФФСФФС	CCCGACCTCC	AAGCCTCAAA	GACATATATC
28/31				TTCGGAGTTT	
	TGGGATAGAC	GCAAAACAAG	GGGC I GGAGG	11CGGAG1-11	CIGINIAIAG
28801			• •	AAGTTGCTAC	
	TACGTCTAAG	TGAGCATATA	CCTTATAAGG	TTCAACGATG	TTACTTTTTT
28851	GCGATCTTTC	CGAAGCCTGG	TTATATGCAA	TCATCTCTGT	TATGGTGTTC
	CGCTAGAAAG	GCTTCGGACC	<b>AATATACGTT</b>	AGTAGAGACA	ATACCACAAG
28901	TGCAGTACCA	TCTTAGCCCT	AGCTATATAT	CCCTACCTTG	ACATTGGCTG
				GGGATGGAAC	
28951	CAACCCAATA	ראייררראיירא	ACCACCCAAC	TTTCCCCGCG	CCCCCTATCC
20331				AAAGGGGCGC	
	CTTGCGTTAT	CTACGGTACT	1661666116	AAAGGGGCGC	GGGCGATACG
					a ma acam
29001				TTGTCCCAGC	
	AAGGTGACGT	TGTTCAACAA	CGGCCGCCGA	AACAGGGTCG	GTTAGTCGGA
			•		
29051				AGCTACTTTA	
	GCGGGTGGAA	GAGGGTGGGG	GTGACTTTAG	TCGATGAAAT	TAGATTGTCC
29101	AGGAGATGAC	TGACACCCTA	GATCTAGAAA	TGGACGGAAT	TATTACAGAG
				ACCTGCCTTA	
20151	CAGCGCCTGC	TAGAAAGACG	CAGGGCAGCG	CCCCACCAAC	AGCGCATGAA
29131				CGGCTCGTTG	
	GTCGCGGACG	MICITICIGE	GICCCGICGC	CGGCTCGTTG	1CGCG1AC11
			mm1 1 0mm001	0010000111	10000m1m0m
29201	TCAAGAGCTC				
	AGTTCTCGAG	GTTCTGTACC	AATTGAACGT	GGTCACGTTT	TCCCCATAGA
29251	TTTGTCTCGT				
•	AAACAGAGCA	TTTCGTCCGG	TTTCAGTGGA	TGCTGTCATT	ATGGTGGCCT
29301	CACCGCCTTA	GCTACAAGTT	GCCAACCAAG	CGTCAGAAAT	TGGTGGTCAT
					ACCACCAGTA
	araccauvy;	COMICAL			

Figure 27 A E

29401	GCTGCATTCA	CTCACCTTGT	CAAGGACCTG	AGGATCTCTG	CACCCTTATT
	CGACGTAAGT	GAGTGGAACA	GTTCCTGGAC	TCCTAGAGAC	GTGGGAATAA
29451	AAGACCCTGT	GCGGTCTCAA	AGATCTTATT	CCCTTTAACT	AATAAAAAA
		CGCCAGAGTT			
29501	AATAATAAAG	CATCACTTAC	TTAAAATCAG	TTAGCAAATT	TCTGTCCAGT
		GTAGTGAATG			•
	IIAIIAIIIC	GIAGIGATIO	millingic	121100111701	noncilooren
29551	<u>ጥጥ አጥጥ</u> ሮ አርርር አ	GCACCTCCTT	CCCCTCCTCC	СУССИСИССИ	ልጥጥርር ልርርጥጥ
23331		CGTGGAGGAA			
	AMIANGICGI	CGIGGWGGW	CGGGAGGAGG	GICGNONCCA	IMPOICON
29601	CCTCCTCCCT	GCAAACTTTC	かたことととなっている	AAATCCAATC	ייירי <u>א</u> כיייייייירירייי
29001		CGTTTGAAAG			
	GGAGGACCGA	CGITIGAMAG	MOGIGIIMON	IIIACCIIAC	AGICAMAGGA
20651	CCMCMMCCMC	TCCATCCGCA	CCCACMAMCM	<b>ጥር</b> እ ጥር ውጥር ውጥ	CCACATCAAC
29651		AGGTAGGCGT			
	GGACAAGGAC	AGGTAGGCGT	GGGTGATAGA	AGIACAACAA	CGICIACTIC
00701		CGTCTGAAGA	m> 00mm0> > 0	CCCCCCCCCA	CAMAMCACAC
29701					
	GCGCGTTCTG	GCAGACTTCT	ATGGAAGTTG	GGGCACATAG	GIAIACIGIG
00054	001110000	00,000	maaammmam	ma concorrecce	mmmcm
29751		CCTCCAACTG			
	CCTTTGGCCA	GGAGGTTGAC	ACGGAAAAGA	ATGAGGAGGG	AAACATAGGG
00001		TCAAGAGAGT	0000000000	ma cococooo	CCCCCMAMCC
29801					
	GGTTACCCAA	AGTTCTCTCA	GGGGGALCCC	ATGAGAGAAA	CGCGGATAGG
20051	0 comem. c	TTACCTCCAA	mccc> mccmm	CCCCTCNNNN	mcccc a a ccc
29851		AATGGAGGTT			
	CTTGGAGATC	AATGGAGGTT	ACCGIACGAA	CGCGAGIIII	ACCCGTTGCC
29901	COMONOMONO	GACGAGGCCG	CC3 3 CCTT3 C	יריייייייייייייייייייייייייייייייייייי	CTARCCACTC
29901		CTGCTCCGGC			
	GGAGAGAGAC	CTGCTCCGGC	CGIIGGAAIG	GAGGGIIIIA	CATTGGTGAC
29951	mc>cccc>cc	TCTCAAAAAA	3.CC3.3.CDC3.3	ACATA A ACCT	CC333T3TCT
29951		AGAGTTTTTT			
	ACTCGGGTGG	AGAGTTTTTT	TGGTTCAGTT	TGTATTTGGA	CCITIATAGA
20001	001000000	C) COM) COMC	>C>>CCCC	NCMCMCCCMC	CCCCCCCACC
30001		CAGTTACCTC			
	CGTGGGGAGT	GTCAATGGAG	TCTTCGGGAT	TGACACCGAC	6666666166
20051	man, , maana	0000001101	C1 Cmc1 CC1 m	0022002020	CCCCCCCTA A
30021	TCTAATGGTC	CGCCCGTTGT			
	AGATTACCAG	CGCCCGTTGT	GIGAGIGGIA	CGITAGIGIC	CGGGGCGAII
20101	CCGTGCACGA	CDCC111CDD	>	CCCAACCACC	CCTC
30101		GAGGTTTGAA			
	GGCACGTGCT	GAGGTTTGAA	TEGTAACGGT	GGGTTCCTGG	GGAGTGTCAC
20171	masassass	100m10000	003110100		CCACCACCCA
30151	TCAGAAGGAA				
	AGTCTTCCTT	TCGATCGGGA	CGTTTGTAGT	CCGGGGAGT	GGIGGIGGCT
		OBB	000000000000000000000000000000000000000	00000000	NOTICO NOTIC
30201	TAGCAGTACC				
	ATCGTCATGG	GAATGATAGT	GACGGAGTGG	GGGAGATTGA	TOACGGTGAC
20055	001000000	03 mm03 0mm2	*********	mmmamacaca	*****
30251	GTAGCTTGGG	GTAACTGAAC			
	CATCGAACCC	GTAACTGAAC	TTTCTCGGGT	AAATATGTGT	TITACCTTTT

Figure 27 AF

30351	TTTGACCGTA	GCAACTGGTC	CAGGTGTGAC	TATTAATAAT	ACTTCCTTGC
	AAACTGGCAT	CGTTGACCAG	GTCCACACTG	ATAATTATTA	TGAAGGAACG
					10.2.00,2.00
20401		TACTGGAGCC	mmcccmmmmc	1000101100	
30401					
	TTTGATTTCA	ATGACCTCGG	AACCCAAAAC	TAAGTGTTCC	GTTATACGTT
30451	CTTAATGTAG	CAGGAGGACT	AAGGATTGAT	TCTCAAAACA	GACGCCTTAT
	CAATTACATC	GTCCTCCTGA	ттсставста	AGAGTTTTGT	CTGCGGAATA
	CARTITATION C				0.0000.
20501		\ CDD\ DOOCD	PPC> PCOPC>	*********	*******
30501		AGTTATCCGT			
	TGAACTACAA	TCAATAGGCA	AACTACGAGT	TTTGGTTGAT	TTAGATTCTG
30551	TAGGACAGGG	CCCTCTTTTT	ATAAACTCAG	CCCACAACTT	GGATATTAAC
	ATCCTGTCCC	GGGAGAAAA	TATTTGAGTC	GGGTGTTGAA	CCTATAATTG
30601	TACAACAAAG	GCCTTTACTT	CTTTACACCT	тсавасавтт	CCAAAAAGCT
30001		CGGAAATGAA			
	ATGITGITTC	CGGAAATGAA	CAAATGTCGA	AGIIIGIIAA	GGITITICGA
30651		CTAAGCACTG			
	ACTCCAATTG	GATTCGTGAC	GGTTCCCCAA	CTACAAACTG	CGATGTCGGT
30701	TAGCCATTAA	TGCAGGAGAT	GGGCTTGAAT	TTGGTTCACC	TAATGCACCA
••••	ATCCCTAATT	ACGTCCTCTA	ССССАВСТТА	AACCAAGTGG	ATTACGTGGT
	nicooin				
20751		CCCTCAAAAC	>>>>	CARCOCCRAC	A A THURST A THUS
30751					
	TTGTGTTTAG	GGGAGTTTTG	TTTTTAACCG	GTACCGGATC	TTAAACTAAG
30801	AAACAAGGCT	ATGGTTCCTA	<b>AACTAGGAAC</b>	TGGCCTTAGT	TTTGACAGCA
	TTTGTTCCGA	TACCAAGGAT	TTGATCCTTG	ACCGGAATCA	AAACTGTCGT
30851	СУССТСССУТ	TACAGTAGGA	מדממממממ	ATCATAACCT	AACTTTCTCC
30031		ATGTCATCCT			
	GICCACGGIA	AIGICAICCI	HIGHTHIAI	IACIAIICGA	TIGARACACC
30901		CTCCATCTCC			
	TGGTGTGGTC	GAGGTAGAGG	ATTGACATCT	GATTTACGTC	TCTTTCTACG
30951	TAAACTCACT	TTGGTCTTAA	CAAAATGTGG	CAGTCAAATA	CTTGCTACAG
	ATTTGAGTGA	AACCAGAATT	GTTTTACACC	GTCAGTTTAT	GAACGATGTC
				•	. •
21001	TTTCAGTTTT	CCCMCMM3	CCC & CTTTCC	CTCCAATATC	TOCALORT
21001					
	AAAGTCAAAA	CCGACAATTT	CCGTCAAACC	GAGGTTATAG	ACCTIGICAA
31051	CAAAGTGCTC				
	GTTTCACGAG	TAGAATAATA	TTCTAAACTG	CTTTTACCTC	ACGATGATTT
	•				
31101	CAATTCCTTC	CTGGACCCAG	AATATTGGAA	CTTTAGAAAT	GGAGATCTTA
J-202		GACCTGGGTC			
	GIIMMOOMMO	aucc10001C	····	MR0310111V	indnri
				63 mmm > 566 5	m
31151	CTGAAGGCAC				
	GACTTCCGTG	TCGGATATGT	TTGCGACAAC	CTAAATACGG	ATTGGATAGT
				· · ·	
31201	GCTTATCCAA	AATCTCACGG	TAAAACTGCC	AAAAGTAACA	TTGTCAGTCA
<del></del>		TTAGAGTGCC			
				<del> </del>	

Figure 27 AG

WO 02/022080 PCT/US01/28861											
31251	AGTTT. A	AACGGAGACA	AAACTAAACC	TGTAACACTA	ATTACAC						
51201			TTTGATTTGG								
31301	TAAACGGTAC	ACAGGAAACA	GGAGACACAA	CTCCAAGTGC	ATACTCTATG						
	ATTTGCCATG	TGTCCTTTGT	CCTCTGTGTT	GAGGTTCACG	TATGAGATAC						
31351	TCATTTTCAT										
			ACCGGTGTTG								
31401	CACATCCTCT										
			GTATGTAACG								
31451	GTGTTATGTT										
			ATAAAAAGTT								
31501			GGGGTGGTGG								
21552											
31551	CGTACCTTAA		TCTTGGGATC								
	GCAIGGAAII	AGIIIGAGIG	1C11GGGA1C	AIAAGI IGGA	CGG1GGAGGG						
31601	TCCCAACACA	CAGAGTACAC	AGTCCTTTCT	CCCCGGCTGG	CCTTAAAAAG						
	AGGGTTGTGT	GTCTCATGTG	TCAGGAAAGA	GGGGCCGACC	GGAATTTTTC						
31651	CATCATATCA	TGGGTAACAG	ACATATTCTT	AGGTGTTATA	TTCCACACGG						
			TGTATAAGAA								
31701	TTTCCTGTCG										
	AAAGGACAGC										
31751	AGCTCACTTA										
			CGACAGGTCG								
31801			CGGGCGGCGA GCCCGCCGCT								
21051			TGCATCAGGA								
31851					CACGACGTCG						
21001	AGCGCGCGAA		•								
31901			GCGCCGCGC								
	100000011	ATTIONCONC	000000000								
31951	CATGGCAGTG	GTCTCCTCAG	CGATGATTCG	CACCGCCCGC	AGCATAAGGC						
	GTACCGTCAC	CAGAGGAGTC	GCTACTAAGC	GTGGCGGGCG	TCGTATTCCG						
32001	GCCTTGTCCT										
			GTCGCGTGGG								
32051	CAGTAACTGC										
					GTGTCACGTT						
32101	GGCGCTGTAT										
					TGCACCGGTA						
32151	CATACCACAA										
	GTATGGTGTT	CGCGTCCATC	TAATTCACCG	CTGGGGAGTA	TTTGTGCGAC						

Figure 27 AH

	_				
32251	CCATATAAAC	CTCTGATTAA	ACATGGCGCC	ATCCACCACC	ATCCTAAACC
		GAGACTAATT			
	001111111111	0	-0111100000		
22201	1000000011	***	000000000000000000000000000000000000000	> CDCC> CCC>	1.000001.0m0
32301		AACCTGCCCG			
	TCGACCGGTT	TTGGACGGGC	GGCCGATATG	TGACGTCCCT	TGGCCCTGAC
32351	GAACAATGAC	AGTGGAGAGC	CCAGGACTCG	TAACCATGGA	TCATCATGCT
	CTTGTTACTG	TCACCTCTCG	GGTCCTGAGC	ATTGGTACCT	AGTAGTACGA
32401	CGTCATGATA	TCAATGTTGG	CACAACACAG	GCACACGTGC	ATACACTTCC
,,,,,,		AGTTACAACC			
	GCAGIACIAI	AG11ACAGCC	0.0110.010	COLOTOCACO	111101011100
22451	man can mana c	AAGCTCCTCC	CCCCMMACAA	CCATATCCCA	CCCNACNACC
32451					
	AGTCCTAATG	TTCGAGGAGG	GCGCAATCTT	GGTATAGGGT	CCCTTGTTGG
32501		TCAGCGTAAA			
	GTAAGGACTT	AGTCGCATTT	AGGGTGTGAC	GTCCCTTCTG	GAGCGTGCAT
32551	ACTCACGTTG	TGCATTGTCA	AAGTGTTACA	TTCGGGCAGC	AGCGGATGAT
	TGAGTGCAAC	ACGTAACAGT	TTCACAATGT	AAGCCCGTCG	TCGCCTACTA
32601	ССТССАСТАТ	GGTAGCGCGG	<b>CTTTCTCTCT</b>	CAAAAGGAGG	TAGACGATCC
32001		CCATCGCGCC			
	GGAGGICAIA	CCATCGCGCC	CAAAGACAGA	GIIIICCICC	AICIGCIAGG
22651	CEL CECEL CC	C1 CTCCCCCC	1010110001	C M M C C M C M M C	CMCCM3 CMCM
32651		GAGTGCGCCG			
	GATGACATGC	CTCACGCGGC	TCTGTTGGCT	CTAGCACAAC	CAGCATCACA
	•				
32701	CATGCCAAAT	GGAACGCCGG	ACGTAGTCAT	ATTTCCTGAA	GCAAAACCAG
	GTACGGTTTA	CCTTGCGGCC	TGCATCAGTA	TAAAGGACTT	CGTTTTGGTC
32751	GTGCGGGCGT	GACAAACAGA	TCTGCGTCTC	CGGTCTCGCC	GCTTAGATCG
	CACGCCCGCA	CTGTTTGTCT	AGACGCAGAG	GCCAGAGCGG	CGAATCTAGC
32801	CTCTGTGTAG	TAGTTGTAGT	ATATCCACTC	TCTCAAAGCA	TCCAGGCGCC
		ATCAACATCA			
	GAGACACATC				
32851		GGGTTCTATG	ሞል እ አርጥርርጥጥ	CATGCCCCCC	тессстерть
32631					
	GGGACCGAAG	CCCAAGATAC	ATTIGAGGAA	GIACGCGGCG	ACGGGACIAI
32901	ACATCCACCA				
	TGTAGGTGGT	GGCGTCTTAT	TCGGTGTGGG	TCGGTTGGAT	GTGTAAGCAA
32951	CTGCGAGTCA				
	GACGCTCAGT	GTGTGCCCTC	CTCGCCCTTC	TCGACCTTCT	TGGTACAAAA
33001	TTTTTTTATT	CCAAAAGATT	ATCCAAAACC	TCAAAATGAA	GATCTATTAA
		GGTTTTCTAA			
	-3				
22051	GTGAACGCGC	TACCA COLOR	<b>ጥርር</b> ርጥርርጥር	<b>ል ል ል ር ጥ ር</b> ጥ ል ር ል	GCCAAAGAAC
22021		AGGGAGGCC			
	CACTIGUGUG	JUUUNAUUUN	ACCUCACCAG	TITOMONIGI	COGILICIIG
			mammaa	macommaca::	********
33101	AGATAATGGC				
	TCTATTACCG	TAAACATTCT	ACAACGTGTT	ACCGAAGGTT	TTCCGTTTGC

Figure 27 AI

33201	CTCTATAAAC	ATTCCAGCAC	CTTCAACCAT	GCCCAAATAA	TTCTCATCTC
	СУСУДУДАТАТС	TAAGGTCGTG	CAACTTCCTA	CCCCTTTATT	AACACTACAC
	GAGAIAIIIG	11110010010	G/2/01/1001/1	COOGILIALI	MONGINGAG
33251		CAATATATCT			•
	CGGTGGAAGA	GTTATATAGA	GATTCGTTTA	GGGCTTATAA	TTCAGGCCGG
33301	ATTGTAAAAA	TCTGCTCCAG	AGCGCCCTCC	ACCTTCAGCC	TCAAGCAGCG
	TAACATTTTT	AGACGAGGTC	TCGCGGGAGG	TGGAAGTCGG	AGTTCGTCGC
•					
33351	3 3 mc 3 mc 3 mm	GCAAAAATTC	N C C TOTO C TOTO N	CACACCTCTA	<b>#####################################</b>
22221					
	TTAGTACTAA	CGTTTTTAAG	TCCAAGGAGT	GICIGGACAI	ATTCTAAGTT
33401		TTAACAAAAA			_
	TTCGCCTTGT	AATTGTTTTT	ATGGCGCTAG	GGCATCCAGG	GAAGCGTCCC
33451	CCAGCTGAAC	ATAATCGTGC	AGGTCTGCAC	GGACCAGCGC	GGCCACTTCC
	GGTCGACTTG	TATTAGCACG	TCCAGACGTG	CCTGGTCGCG	CCGGTGAAGG
	***************************************				
33501	CCCCCACCAA	CCATGACAAA	ACAACCCACA	CTCATTATCA	САСССАТАСТ
33301		GGTACTGTTT		_	
	GGCGGTCCTT	GGIACIGITI	1011666161	GACIAAIACI	GIGCGIAIGA
33551	•	CTAACCAGCG			
	GCCTCGATAC	GATTGGTCGC	ATCGGGGCTA	CATTCGAACA	ACGTACCCGC
33601	GCGATATAAA	ATGCAAGGTG	CTGCTCAAAA	AATCAGGCAA	AGCCTCGCGC
	CGCTATATTT	TACGTTCCAC	GACGAGTTTT	TTAGTCCGTT	TCGGAGCGCG
33651	AAAAAGAAA	GCACATCGTA	GTCATGCTCA	TGCAGATAAA	GGCAGGTAAG
		CGTGTAGCAT			
	1111110111	COTOTAGCAT	CAGIACOAGI	710010111111	
33701	CMCCCC NACC	ACCACAGAAA	33C3C3CC3T	<del>መመ</del> መጥር ምር ጥር እ	አአርአጥርጥርጥር
33/01					
	GAGGCCTTGG	TGGTGTCTTT	TTCTGTGGTA	AAAAGAGAGT	TTGTACAGAC
33751		CATAAACACA			
	GCCCAAAGAC	GTATTTGTGT	TTTATTTAT	TGTTTTTTTG	TAAATTTGTA
33801	TAGAAGCCTG	TCTTACAACA	GGAAAAACAA	CCCTTATAAG	CATAAGACGG
	ATCTTCGGAC	AGAATGTTGT	CCTTTTTGTT	GGGAATATTC	GTATTCTGCC
33851	ACTACGGCCA	TGCCGGCGTG	ACCGTAAAAA	AACTGGTCAC	CGTGATTAAA
		ACGGCCGCAC			
	IGNIGCCOGi	ACGGCCGCAC	100CA11111	110000000	ocne man
22001	AAGCACCACC	C) C) CCCCCC	CCCCCACCACC	CCCACMCAMA	3 mcm3 3 C3 Cm
33301					
	TTCGTGGTGG	CTGTCGAGGA	GCCAGTACAG	GCCTCAGTAT	TACATTCTGA
33951	CGGTAAACAC				
	GCCATTTGTG	TAGTCCAACT	AAGTGTAGCC	AGTCACGATT	TTTCGCTGGC
34001	AAATAGCCCG	GGGGAATACA	TACCCGCAGG	CGTAGAGACA	ACATTACAGC
		CCCCTTATGT			
34051	CCCCATAGGA	ССТАТААСАА	בסבתבבתתבב	ACACAAAAAC	ACATAAACAC
34031		CCATATTGTT			
	GGGGTATCCT	CCMIMITGIT	TIMMITMICC	1010111110	1914111010

Figure 27AJ

WO 02/02	WO 02/022080 PCT/US01/28861											
34151	ACATACHECG TGTATGTCGC	CTTCCACAGC GAAGGTGTCG										
34201	AAAAGAAAAC TTTTCTTTTG	CTATTAAAAA GATAATTTTT										
34251	AGTCACAGTG TCAGTGTCAC	TAAAAAAGGG ATTTTTTCCC			· · · -							
34301	AAAAATGACG TTTTTACTGC	TAACGGTTAA ATTGCCAATT										
34351	GCGAACCTAC CGCTTGGATG	GCCCAGAAAC CGGGTCTTTG										
34401	TCGTCACTTC AGCAGTGAAG	CGTTTTCCCA GCAAAAGGGT										
34451	ACAATTCCCA TGTTAAGGGT	ACACATACAA TGTGTATGTT										
34501	CCCCGTTCCC GGGGCAAGGG	ACGCCCCGCG TGCGGGGCGC										
					PacI							
34551	ATATTGGCTT TATAACCGAA	CAATCCAAAA GTTAGGTTTT										
34601	AATTCGGATC TTAAGCCTAG	TGCGACGCGA ACGCTGCGCT										
34651	CTCGCTTCCG GAGCGAAGGC	GCGGCATCGG CGCCGTAGCC										
34701	GCAGGTAGAT CGTCCATCTA	GACGACCATC CTGCTGGTAG	=									
34751	GGAACCGTAA CCTTGGCATT	AAAGGCCGCG TTTCCGGCGC										
34801	CCTGACGAGC GGACTGCTCG	ATCACAAAAA TAGTGTTTTT		· · -								
34851	GACAGGACTA CTGTCCTGAT	TAAAGATACC ATTTCTÄTGG										
34901	GCTCTCCTGT CGAGAGGACA	TCCGACCCTG AGGCTGGGAC										
34951	CCTTCGGGAA GGAAGCCCTT	GCGTGGCGCT CGCACCGCGA										
35001												

Figure 27 AK

	AAGTCGGGCT	GGCGACGCGG	AATAGGCCAT	TGATAGCAGA	ACTCAGGTTG
35101	CCCCTAAGAC	ACCACTTATC	CCCACTGGCA	GCAGCCACTG	GTAACAGGAT
33101				CGTCGGTGAC	
	GGCCATTCTG	TGCTGAATAG	CGGTGACCGT	COTCGGTGAC	CATTGTCCTA
35151	TAGCAGAGCG	AGGTATGTAG	GCGGTGCTAC	AGAGTTCTTG	AAGTGGTGGC
	ATCGTCTCGC	TCCATACATC	CGCCACGATG	TCTCAAGAAC	TTCACCACCG
35201	CTAACTACGG	CTACACTAGA	AGGACAGTAT	TTGGTATCTG	CGCTCTGCTG
	GATTGATGCC	GATGTGATCT	TCCTGTCATA	AACCATAGAC	GCGAGACGAC
35251	አአርርርእርጥፕአ	CCTTCCCAAA	AACACTTCCT	AGCTCTTGAT	CCGCCAAACA
77271				TCGAGAACTA	
	TICGGICAAT	GGAAGCCTTT	TICICAACCA	TCGAGAACTA	GGCCGIIIGI
35301	AACCACCGCT	GGTAGCGGTG	GTTTTTTTGT	TTGCAAGCAG	CAGATTACGC
	TTGGTGGCGA	CCATCGCCAC	CAAAAAAACA	AACGTTCGTC	GTCTAATGCG
35351	•••••			TGATCTTTTC	
	CGTCTTTTTT	TCCTAGAGTT	CTTCTAGGAA	ACTAGAAAAG	ATGCCCCAGA
35401	CACCCTCACT	GCAACGAAAA	CTCACGTTAA	GGGATTTTGG	TCATGAGATT
33401			-	CCCTAAAACC	
	CIGCGAGICA	CCTIGCTTT	GAGIGCAAII	CCCTAAAACC	AGIACICIAA
35451	ATCAAAAAGG	ATCTTCACCT	AGATCCTTTT	AAATCAATCT	AAAGTATATA
	TAGTTTTTCC	TAGAAGTGGA	TCTAGGAAAA	TTTAGTTAGA	TTTCATATAT
25501				CTTAATCAGT	C) CCC) CCM)
35501					
	ACTCATTTGA	ACCAGACTGT	CAATGGTTAC	GAATTAGTCA	CTCCGTGGAT
35551	TCTCAGCGAT	CTGTCTATTT	CGTTCATCCA	TAGTTGCCTG	ACTCCCCGTC
				ATCAACGGAC	
35601	GTGTAGATAA	CTACGATACG	GGAGGGCTTA	CCATCTGGCC	CCAGTGCTGC
	CACATCTATT	GATGCTATGC	CCTCCCGAAT	GGTAGACCGG	GGTCACGACG
35651	AATCATACCC	CCACACCCAC	GCTCACCGGC	TCCAGATTTA	TCAGCAATAA
33031				AGGTCTAAAT	
	TTACTATGGC	GCTCTGGGTG	CGAGIGGCCG	AGGICIAAAI	AGICGIIAII
35701	ACCAGCCAGC				
	TGGTCGGTCG	GCCTTCCCGG	CTCGCGTCTT	CACCAGGACG	TTGAAATAGG
25751	00000000000	3.0m0m3.mm3.5	BWCBWCCCC	CAACCMACAC	TAAGTAGTTC
32/21					
	CGGAGGTAGG	TCAGATAATT	AACAACGGCC	CTTCGATCTC	ATTCATCAAG
35801	GCCAGTTAAT	ACTTTCCCCA	ACGTTGTTGC	CATTGCTACA	GGCATCGTGG
JJ001				GTAACGATGT	
35851	TGTCACGCTC	GTCGTTTGGT	ATGGCTTCAT	TCAGCTCCGG	TTCCCAACGA
				AGTCGAGGCC	
25001	max + 0000010	MM3 C3 MC3 MC		MCC233355	CGGTTAGCTC
33901					
	AGTTCCGCTC	AATGTACTAG	GGGGTACAAC	ACGITTITTC	GCCAATCGAG
35951	СТТСССТССТ	CCGATCGTTG	TCAGAAGTAA	GTTGGCCGCA	GTGTTATCAC
					CACAATAGTG
	ADDOC CAGO				

Figure 2 7AL

					_
36051	AGATGCTTTT	CTGTGACTGG	TGAGTACTCA	ACCAAGTCAT	TCTGAGAATA
	TCTACGAAAA	GACACTGACC	ACTCATGAGT	TGGTTCAGTA	AGACTCTTAT
36101	GTGTATGCGG	CGACCGAGTT	GCTCTTGCCC	GGCGTCAACA	CGGGATAATA
	CACATACGCC	GCTGGCTCAA	CGAGAACGGG	CCGCAGTTGT	GCCCTATTAT
36151	CCGCGCCACA	TAGCAGAACT	TTAAAAGTGC	TCATCATTGG	AAAACGTTCT
	GGCGCGGTGT	ATCGTCTTGA	AATTTTCACG	AGTAGTAACC	TTTTGCAAGA
36201	TCGGGGCGAA	AACTCTCAAG	GATCTTACCG	CTGTTGAGAT	CCAGTTCGAT
	AGCCCCGCTT	TTGAGAGTTC	CTAGAATGGC	GACAACTCTA	GGTCAAGCTA
36251		CGTGCACCCA			
	CATTGGGTGA	GCACGTGGGT	TGACTAGAAG	TCGTAGAAAA	TGAAAGTGGT
36301		GTGAGCAAAA			•
	CGCAAAGACC	CACTCGTTTT	TGTCCTTCCG	TTTTACGGCG	TTTTTTCCCT
				> <b>6</b> > C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b> C <b>6</b>	
36351		CACGGAAATG			
	TATTCCCGCT	GTGCCTTTAC	AACTTATGAG	TATGAGAAGG	AAAAAGTTAT
36401	mm s mm c s s c c	ATTTATCAGG	ርጥጥ አጥጥርጥርጥ	CATCACCCCA	ጥልሮልጥልጥጥጥር
36401		TAAATAGTCC			
	AMIMACIICG	TAMATAGICC	CANTANCAGA	GIACICGCCI	NIGINIAMC
36451	<b>ል አጥር</b> ጥልጥጥጥ ል	GAAAAATAAA	CAAATAGGGG	TTCCGCGCAC	ATTTCCCCGA
20427		CTTTTTATTT			
			0		
36501	AAAGTGCCAC	CTGACGTCTA	AGAAACCATT	ATTATCATGA	CATTAACCTA
		GACTGCAGAT			
36551	TAAAAATAGG	CGTATCACGA	GGCCCTTTCG	TCTTCAAGAA	TTGGATCCGA
	ATTTTTATCC	GCATAGTGCT	CCGGGAAAGC	AGAAGTTCTT	AACCTAGGCT

## PacI

36601 ATTCTTAATT TCTTAATTAA (SEQ ID NO:34)
TAAGAATTAA AGAATTAATT (SEQ ID NO:35)

Ingure 27 AM

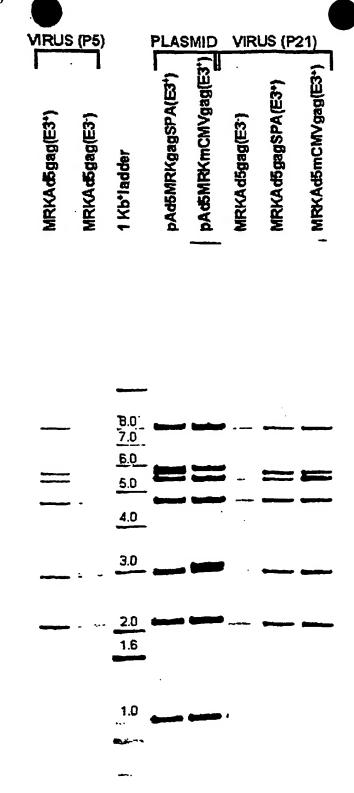


FIGURE 28

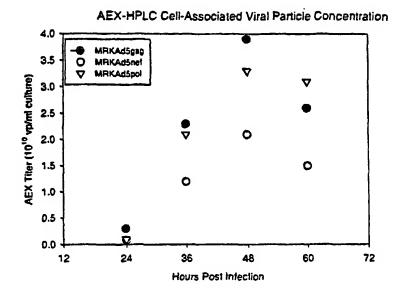


FIGURE 29A

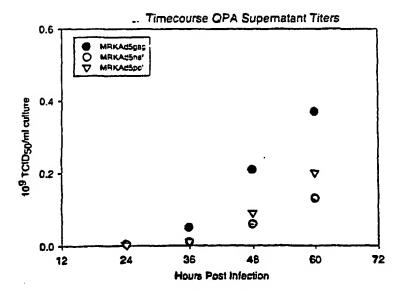


FIGURE 29B

Figure 30'A"

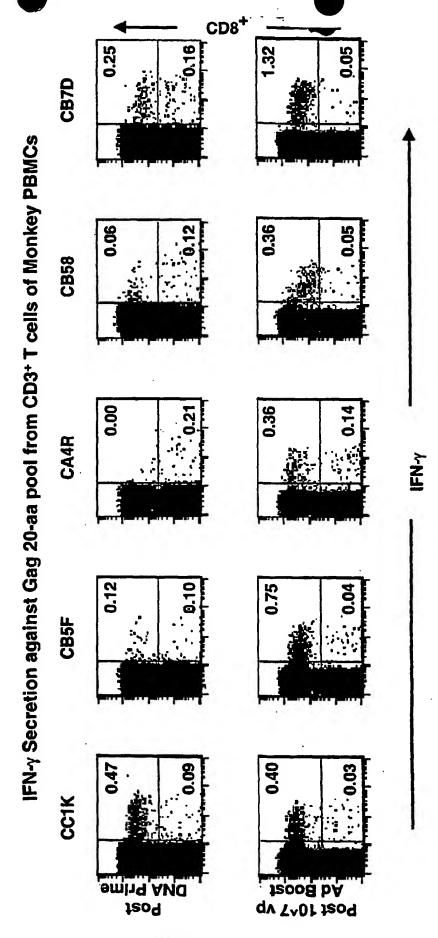
Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys

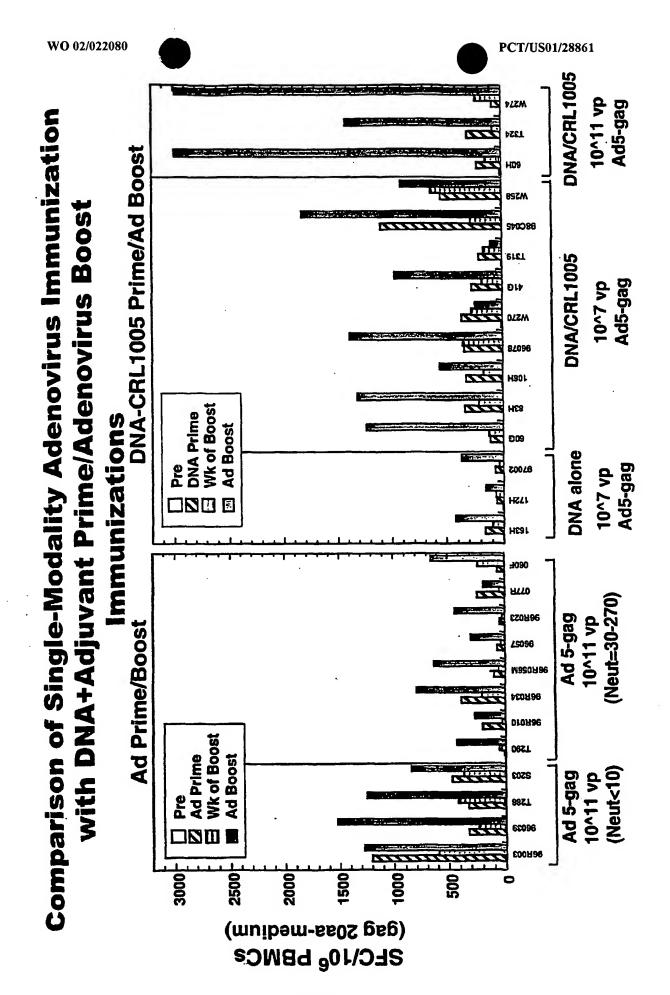
245

agg Arg	tgg Trp	atc Ile	atc Ile 260	ctg Leu	ggc Gly	ctg Leu	aac Asn	aag Lys 265	att Ile	gtg Val	agg Arg	atg Met	tac Tyr 270	tcc Ser	ccc Pro	816
acc Thr	tcc Ser	atc Ile 275	ctg Leu	gac Asp	atc Ile	agg Arg	cag Gln 280	ggc Gly	ccc Pro	aag Lys	gag Glu	ccc Pro 285	ttc Phe	agg Arg	gac Asp	864
tat Tyr	gtg Val 290	gac Asp	agg Arg	ttc Phe	tac Tyr	aag Lys 295	acc Thr	ctg Leu	agg Arg	gct Ala	gag Glu 300	cag Gln	gcc Ala	tcc Ser	cag Gln	912
													aat Asn			960
													gcc Ala			1008
gag Glu	gag Glu	atg Met	atg Met 340	aca Thr	gcc Ala	tgc Cys	cag Gln	999 Gly 345	gtg Val	GJA aaa	ggc Gly	cct Pro	ggt Gly 350	cac His	aag Lys	1056
													tcc Ser			1104
													aca Thr			1152
	Phe												tgt Cys			1200
													cac His			1248
													atc Ile 430			1296
													cct Pro			1344
aca Thr	gcc Ala 450	cct Pro	ccc Pro	gag Glu	gag Glu	tcc Ser 455	ttc Phe	agg <b>Ar</b> g	ttť Phe	ejà ââā	gag Glu 460	gag Glu	aag Lys	acc	acc Thr	1392
													Pro			1440
tcc Ser	ctg Leu	agg Arg	tcc Ser	ctg Leu 485	ttt Phe	ggc Gly	aac Asn	gac Asp	ccc Pro 490	tcc Ser	tcc Ser	cag Gln	taa *	(SI	D NO:36) D NO:37)	1482

Figure 30 B

Figure 31







ATGGGTGCTA	GGGCTTCTGT	GCTGTCTGGT	GGTGAGCTGG	ACAAGTGGGA	GAAGATCAGG
CTGAGGCCTG	GTGGCAAGAA	GAAGTACAAG	CTAAAGCACA	TTGTGTGGGC	CTCCAGGGAG
CTGGAGAGGT	TTGCTGTGAA	CCCTGGCCTG	CTGGAGACCT	CTGAGGGGTG	CAGGCAGATC
CTGGGCCAGC	TCCAGCCCTC	CCTGCAAACA	GGCTCTGAGG	AGCTGAGGTC	CCTGTACAAC
ACAGTGGCTA	CCCTGTACTG	TGTGCACCAG	AAGATTGATG	TGAAGGACAC	CAAGGAGGCC
CTGGAGAAGA	TTGAGGAGGA	GCAGAACAAG	TCCAAGAAGA	AGGCCCAGCA	GGCTGCTGCT
GGCACAGGCA	ACTCCAGCCA	GGTGTCCCAG	AACTACCCCA	TTGTGCAGAA	CCTCCAGGGC
CAGATGGTGC	ACCAGGCCAT	CTCCCCCGG	ACCCTGAATG	CCTGGGTGAA	GGTGGTGGAG
GAGAAGGCCT	TCTCCCCTGA	GGTGATCCCC	ATGTTCTCTG	CCCTGTCTGA	GGGTGCCACC
CCCCAGGACC	TGAACACCAT	GCTGAACACA	GTGGGGGCC	ATCAGGCTGC	CATGCAGATG
CTGAAGGAGA	CCATCAATGA	GGAGGCTGCT	GAGTGGGACA	GGCTGCATCC	TGTGCACGCT
GGCCCCATTG	CCCCGGCCA	GATGAGGGAG	CCCAGGGGCT	CTGACATTGC	TGGCACCACC
TCCACCCTCC	AGGAGCAGAT	TGGCTGGATG	ACCAACAACC	CCCCCATCCC	TGTGGGGGAA
	GGTGGATCAT				
TCCATCCTGG	ACATCAGGCA	GGGCCCCAAG	GAGCCCTTCA	GGGACTATGT	GGACAGGTTC
TACAAGACCC	TGAGGGCTGA	GCAGGCCTCC	CAGGAGGTGA	AGAACTGGAT	GACAGAGACC
CTGCTGGTGC	AGAATGCCAA	CCCTGACTGC	AAGACCATCC	TGAAGGCCCT	GGGCCCTGCT
GCCACCCTGG	AGGAGATGAT	GACAGCCTGC	CAGGGGGTGG	GGGGCCCTGG	TCACAAGGCC
AGGGTGCTGG	CTGAGGCCAT	GTCCCAGGTG	ACCAACTCCG	CCACCATCAT	GATGCAGAGG
	GGAACCAGAG				
ATTGCCAAGA	ACTGTAGGGC	CCCCAGGAAG	AAGGGCTGCT	GGAAGTGTGG	CAAGGAGGGC
CACCAGATGA	AGGACTGCAA	TGAGAGGCAG	GCCAACTTCC	TGGGCAAAAT	CTGGCCCTCC
CACAAGGGCA	GGCCTGGCAA	CTTCCTCCAG	TCCAGGCCTG	AGCCCACAGC	CCCTCCCGAG
					GCCCATTGAC
					CTCCTCCCAG
•					CATGGATGGC
					GGAAATCTGC
_					CTACAACACC
_					GGACTTCAGG
					CCACCCCGCT
					CTTCTCTGTG
					CAACAATGAG
					CTCCCTGCC
=					CCCTGACATT
					TGGGCAGCAC
					CACCCCTGAC
					CCCCGACAAG
					TGACATCCAG
					A GGTGAGGCAG
					r Gactgaggag
GCTGAGCTGG	AGCTGGCTGA	GAACAGGGAG	ATCCTGAAGO	AGCCTGTGC	A TGGGGTGTAC

## FIGURE 33B

TATGACCCCT CCAAGGACCT GATTGCTGAG ATCCAGAAGC AGGGCCAGGG CCAGTGGACC TACCAAATCT ACCAGGAGCC CTTCAAGAAC CTGAAGACTG GCAAGTATGC CAGGATGAGG GGGGCCCACA CCAATGATGT GAAGCAGCTG ACTGAGGCTG TGCAGAAGAT CACCACTGAG TCCATTGTGA TCTGGGGCAA GACCCCCAAG TTCAAGCTGC CCATCCAGAA GGAGACCTGG GAGACCTGGT GGACTGAGTA CTGGCAGGCC ACCTGGATCC CTGAGTGGGA GTTTGTGAAC ACCCCCCCC TGGTGAAGCT GTGGTACCAG CTGGAGAAGG AGCCCATTGT GGGGGCTGAG ACCITCIATG TGGCTGGGGC TGCCAACAGG GAGACCAAGC TGGGCAAGGC TGGCTATGTG ACCAACAGGG GCAGGCAGAA GGTGGTGACC CTGACTGACA CCACCAACCA GAAGACTGCC CTCCAGGCCA TCTACCTGGC CCTCCAGGAC TCTGGCCTGG AGGTGAACAT TGTGACTGCC TCCCAGTATG CCCTGGGCAT CATCCAGGCC CAGCCTGATC AGTCTGAGTC TGAGCTGGTG AACCAGATCA TTGAGCAGCT GATCAAGAAG GAGAAGGTGT ACCTGGCCTG GGTGCCTGCC CACAAGGGCA TTGGGGGCAA TGAGCAGGTG GACAAGCTGG TGTCTGCTGG CATCAGGAAG GTGCTGTTCC TGGATGGCAT TGACAAGGCC CAGGATGAGC ATGAGAAGTA CCACTCCAAC TGGAGGGCTA TGGCCTCTGA CTTCAACCTG CCCCCTGTGG TGGCTAAGGA GATTGTGGCC TCCTGTGACA AGTGCCAGCT GAAGGGGGAG GCCATGCATG GGCAGGTGGA CTGCTCCCCT GGCATCTGGC AGCTGGCCTG CACCCACCTG GAGGGCAAGG TGATCCTGGT GGCTGTGCAT GTGGCCTCCG GCTACATTGA GGCTGAGGTG ATCCCTGCTG AGACAGGCCA GGAGACTGCC TACTTCCTGC TGAAGCTGGC TGGCAGGTGG CCTGTGAAGA CCATCCACAC TGCCAATGGC TCCAACTTCA CTGGGGCCAC AGTGAGGGCT GCCTGCTGGT GGGCTGGCAT CAAGCAGGAG TTTGGCATCC CCTACAACCC CCAGTCCCAG GGGGTGGTGG CCTCCATGAA CAAGGAGCTG AAGAAGATCA TTGGGCAGGT GAGGGACCAG GCTGAGCACC TGAAGACAGC TGTGCAGATG GCTGTGTTCA TCCACAACTT CAAGAGGAAG GGGGGCATCG GGGGCTACTC CGCTGGGGAG AGGATTGTGG ACATCATTGC CACAGACATC CAGACCAAGG AGCTCCAGAA GCAGATCACC AAGATCCAGA ACTTCAGGGT GTACTACAGG GACTCCAGGA ACCCCCTGTG GAAGGGCCCT GCCAAGCTGC TGTGGAAGGG GGAGGGGGGT GTGGTGATCC AGGACAACTC TGACATCAAG GTGGTGCCCA GGAGGAAGGC CAAGATCATC AGGGACTATG GCAAGCAGAT GGCTGGGGAT GACTGTGTGG CCTCCAGGCA GGATGAGGAC TAA SEQ ID NO: 38

## FIGURE 34A

Met Gly Ala Arg Ala Ser Val Leu Ser Gly Gly Glu Leu Asp Lys Trp Glu Lys Ile Arg Leu Arg Pro Gly Gly Lys Lys Lys Tyr Lys Leu Lys His Ile Val Trp Ala Ser Arg Glu Leu Glu Arg Phe Ala Val Asn Pro Gly Leu Leu Glu Thr Ser Glu Gly Cys Arg Gln Ile Leu Gly Gln Leu Gln Pro Ser Leu Gln Thr Gly Ser Glu Glu Leu Arg Ser Leu Tyr Asn Thr Val Ala Thr Leu Tyr Cys Val His Gln Lys Ile Asp Val Lys Asp Thr Lys Glu Ala Leu Glu Lys Ile Glu Glu Glu Glu Asn Lys Ser Lys Lys Lys Ala Gln Gln Ala Ala Gly Thr Gly Asn Ser Ser Gln Val Ser Gln Asn Tyr Pro Ile Val Gln Asn Leu Gln Gly Gln Met Val His Gln Ala Ile Ser Pro Arg Thr Leu Asn Ala Trp Val Lys Val Val Glu Glu Lys Ala Phe Ser Pro Glu Val Ile Pro Met Phe Ser Ala Leu Ser Glu Gly Ala Thr Pro Gln Asp Leu Asn Thr Met Leu Asn Thr Val Gly Gly His Gln Ala Ala Met Gln Met Leu Lys Glu Thr Ile Asn Glu Glu Ala Ala Glu Trp Asp Arg Leu His Pro Val His Ala Gly Pro Ile Ala Pro Gly Gln Met Arg Glu Pro Arg Gly Ser Asp Ile Ala Gly Thr Thr Ser Thr Leu Gln Glu Gln Ile Gly Trp Met Thr Asn Asn Pro Pro Ile Pro Val Gly Glu Ile Tyr Lys Arg Trp Ile Ile Leu Gly Leu Asn Lys Ile Val Arg Met Tyr Ser Pro Thr Ser Ile Leu Asp Ile Arg Gln Gly Pro Lys Glu Pro Phe Arg Asp Tyr Val Asp Arg Phe Tyr Lys Thr Leu Arg Ala Glu Gln Ala Ser Gln Glu Val Lys Asn Trp Met Thr Glu Thr Leu Leu Val Gln Asn Ala Asn Pro Asp Cys Lys Thr Ile Leu Lys Ala Leu Gly Pro Ala Ala Thr Leu Glu Glu Met Met Thr Ala Cys Gln Gly Val Gly Gly Pro Gly His Lys Ala Arg Val Leu Ala Glu Ala Met Ser Gln Val Thr Asn Ser Ala Thr Ile Met Met Gln Arg Gly Asn Phe Arg Asn Gln Arg Lys Thr Val Lys Cys Phe Asn Cys Gly Lys Val Gly His Ile Ala Lys Asn Cys Arg Ala Pro Arg Lys Lys Gly Cys Trp Lys Cys Gly Lys Glu Gly His Gln Met Lys Asp Cys Asn Glu Arg Gln Ala Asn Phe Leu Gly Lys Ile Trp Pro Ser His Lys Gly Arg Pro Gly Asn Phe Leu Gln Ser Arg Pro Glu Pro Thr Ala Pro Pro Glu Glu Ser Phe Arg Phe Gly Glu Glu Lys Thr Thr Pro Ser Gln Lys Gln Glu Pro Ile Asp Lys Glu Leu Tyr Pro Leu Ala Ser Leu Arg Ser Leu Phe Gly Asn Asp Pro Ser Ser Gln Met Ala Pro Ile Ser Pro Ile Glu Thr Val Pro Val Lys Leu Lys Pro Gly Met Asp Gly Pro Lys Val Lys Gln Trp Pro Leu Thr Glu Glu Lys Ile Lys Ala Leu Val Glu Ile Cys Thr Glu Met Glu Lys Glu Gly Lys Ile Ser Lys Ile Gly Pro Glu Asn Pro Tyr Asn Thr Pro Val Phe Ala Ile Lys Lys Lys Asp Ser Thr Lys Trp Arg Lys Leu Val Asp Phe Arg Glu Leu Asn Lys Arg Thr Gln Asp Phe Trp Glu Val Gln Leu Gly Ile Pro His Pro Ala Gly Leu Lys Lys Lys Ser Val Thr Val Leu Ala Val Gly Asp Ala Tyr Phe Ser Val Pro Leu Asp Glu Asp Phe Arg Lys Tyr Thr Ala Phe Thr Ile Pro Ser Ile Asn Asn Glu Thr Pro Gly Ile Arg Tyr Gln Tyr Asn Val Leu Pro Gln Gly Trp Lys Gly Ser Pro Ala Ile Phe Gln Ser Ser Met Thr Lys Ile Leu Glu Pro Phe Arg Lys Gln Asn Pro Asp Ile Val Ile Tyr Gln Tyr Met Ala Ala Leu Tyr Val Gly Ser Asp Leu Glu Ile Gly Gln His Arg Thr Lys Ile Glu Glu Leu Arg Gln His Leu Leu Arg Trp Gly Leu Thr Thr Pro Asp Lys Lys His Gln Lys Glu Pro Pro Phe Leu Trp Met Gly Tyr Glu Leu His Pro

## FIGURE 34B

Asp Lys Trp Thr Val Gln Pro Ile Val Leu Pro Glu Lys Asp Ser Trp Thr Val Asn Asp Ile Gln Lys Leu Val Gly Lys Leu Asn Trp Ala Ser Gln Ile Tyr Pro Gly Ile Lys Val Arg Gln Leu Cys Lys Leu Leu Arg Gly Thr Lys Ala Leu Thr Glu Val Ile Pro Leu Thr Glu Glu Ala Glu Leu Glu Leu Ala Glu Asn Arg Glu Ile Leu Lys Glu Pro Val His Gly Val Tyr Tyr Asp Pro Ser Lys Asp Leu Ile Ala Glu Ile Gln Lys Gln Gly Gln Gly Gln Trp Thr Tyr Gln Ile Tyr Gln Glu Pro Phe Lys Asn Leu Lys Thr Gly Lys Tyr Ala Arg Met Arg Gly Ala His Thr Asn Asp Val Lys Gln Leu Thr Glu Ala Val Gln Lys Ile Thr Thr Glu Ser Ile Val Ile Trp Gly Lys Thr Pro Lys Phe Lys Leu Pro Ile Gln Lys Glu Thr Trp Glu Thr Trp Trp Thr Glu Tyr Trp Gln Ala Thr Trp Ile Pro Glu Trp Glu Phe Val Asn Thr Pro Pro Leu Val Lys Leu Trp Tyr Gln Leu Glu Lys Glu Pro Ile Val Gly Ala Glu Thr Phe Tyr Val Ala Gly Ala Ala Asn Arg Glu Thr Lys Leu Gly Lys Ala Gly Tyr Val Thr Asn Arg Gly Arg Gln Lys Val Val Thr Leu Thr Asp Thr Thr Asn Gln Lys Thr Ala Leu Gln Ala Ile Tyr Leu Ala Leu Gln Asp Ser Gly Leu Glu Val Asn Ile Val Thr Ala Ser Gln Tyr Ala Leu Gly Ile Ile Gln Ala Gln Pro Asp Gln Ser Glu Ser Glu Leu Val Asn Gln Ile Ile Glu Gln Leu Ile Lys Lys Glu Lys Val Tyr Leu Ala Trp Val Pro Ala His Lys Gly Ile Gly Gly Asn Glu Gln Val Asp Lys Leu Val Ser Ala Gly Ile Arg Lys Val Leu Phe Leu Asp Gly Ile Asp Lys Ala Gln Asp Glu His Glu Lys Tyr His Ser Asn Trp Arg Ala Met Ala Ser Asp Phe Asn Leu Pro Pro Val Val Ala Lys Glu Ile Val Ala Ser Cys Asp Lys Cys Gln Leu Lys Gly Glu Ala Met His Gly Gln Val ASD Cys Ser Pro Gly Ile Trp Gln Leu Ala Cys Thr His Leu Glu Gly Lys Val Ile Leu Val Ala Val His Val Ala Ser Gly Tyr Ile Glu Ala Glu Val Ile Pro Ala Glu Thr Gly Gln Glu Thr Ala Tyr Phe Leu Leu Lys Leu Ala Gly Arg Trp Pro Val Lys Thr Ile His Thr Ala Asn Gly Ser Asn Phe Thr Gly Ala Thr Val Arg Ala Ala Cys Trp Trp Ala Gly Ile Lys Gln Glu Phe Gly Ile Pro Tyr Asn Pro Gln Ser Gln Gly Val Val Ala Ser Met Asn Lys Glu Leu Lys Lys Ile Ile Gly Gln Val Arg Asp Gln Ala Glu His Leu Lys Thr Ala Val Gln Met Ala Val Phe Ile His Asn Phe Lys Arg Lys Gly Gly Lle Gly Gly Tyr Ser Ala Gly Glu Arg Ile Val Asp Ile Ile Ala Thr Asp Ile Gln Thr Lys Glu Leu Gln Lys Gln Ile Thr Lys Ile Gln Asn Phe Arg Val Tyr Tyr Arg Asp Ser Arg Asn Pro Leu Trp Lys Gly Pro Ala Lys Leu Leu Trp Lys Gly Glu Gly Ala Val Val Ile Gln Asp Asn Ser Asp Ile Lys Val Val Pro Arg Arg Lys Ala Lys Ile Ile Arg Asp Tyr Gly Lys Gln Met Ala Gly Asp Asp Cys Val Ala Ser Arg Gln Asp Glu Asp SEQ ID NO: 39

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IPC(7) US CL According to B. FIEL Minimum do	SSIFICATION OF SUBJECT MATTER  : C12N 15/86 : 435/456  International Patent Classification (IPC) or to both no IDS SEARCHED  cumentation searched (classification system followed 24/205.1, 207.1, 227.1, 233.1; 435/69.1, 69.3, 173.	by classification symbols)	
Documentati	on searched other than minimum documentation to the	e extent that such documents are included	l in the fields searched
	ata base consulted during the international search (nan continuation Sheet	ne of data base and, where practicable, s	earch terms used)
C. DOC	UMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where ap	propriate, of the relevant passages	Relevant to claim No.
X	WO 96/39178 (ERTL et al.) 12 December 1996 (12		1-3, 8-11, 18
Y Y	and claims 1 and 5.		4, 5, 13-17, 29-32, 34, 35, 37
X 	US 6,019,978 A (ERTL et al.) 1 February 2000, (01	/02/2000), see columns 2, 7 and 8.	1-3, 8-11, 18
Y			4, 5, 13-17, 29-32, 34, 35, 37
X,P	US 6,287,571 6 (ERTL et al.) 11 September 200 and claim 1.	01 (11/09/2001), see columns 2, 7, 8	1, 9, 18
X	US 5,643,579A (HUNG et al.) 1 July 1997 (01/07/	1997), see examples 1, 2, 25 and 26.	1-3, 8, 9-11, 18
Y	,		4,5,13-17, 29-32, 34, 35, 37
Y	WANG et al. The use of an E1-deleted, replication expressing the rabies virus glycoprotein for early vi Journal of Virology (March 1997) Vol. 71, No. 5, 1	accination of mice against rabies virus.	1-3, 9-11, 13-18
Further	documents are listed in the continuation of Box C.	See patent family annex.	
	pecial categories of cited documents:	"T" later document published after the in	ternational filing date or
	t defining the general state of the art which is not considered to ticular relevance	priority date and not in conflict with understand the principle or theory us	the application but cited to aderlying the invention
"E" earlier ap	pplication or patent published on or after the international filing	"X" document of particular relevance; the considered novel or cannot be considered movel or cannot be considered when the document is taken alor	dered to involve an inventive
	t which may throw doubts on priority claim(s) or which is cited ish the publication date of another citation or other special reason fled)	"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art	
"O" documen	ment referring to an oral disclosure, use, exhibition or other means		
	t published prior to the international filing date but later than the	"&" document member of the same paten	ı ramıry
Date of the	actual completion of the international search	Date of mailing of the international sea	arch report
	2002 (06.02.2002) uailing address of the ISA/US	Authorized officer	1.10
Cox Box	missioner of Patents and Trademarks	Ulrike Winkler, Ph.D.	aldkins for
	o. (703)305-3230	Telephone No. 703-308-0196	[]

Form PCT/ISA/210 (second sheet) (July 1998)



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ategory *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
<b>Y</b>	NATUK et al. Immunogenicity of recombinant human adenovirus -human immunodeficiency virus vaccines in chimpanzees. Aids Research and Human Retroviruses (1993) Vol. 9, No. 5, pp395-404, see material and methods.	1, 9, 29-32
Y	PREVEC et al. Immune response to HIV-1 gag antigens induced by recombinant adenovirus vectors in mice and rhesus macaque monkeys. Journal of Acquired Immune Deficincy Syndrome. (1991) Vol. 4, No. 6 pp. 568-76, see abstract.	1, 9, 29-32
Y	LORI et al. Rapid protection against human immunodeficiency virus type 1 (HIV-1) replication mediated by high efficiency non-retroviral delivery of genes interfering with HIV-1 tat and gag. Gene Therapy (1994) Vol. 1, No. 1, pp. 27-31, see abstract.	1, 9
Y	PFARR et al. Differential effects of polyadenylation regions on gene expression in mammalian cells. DNA (1986) Vol. 5, No. 2, pp.115-22, see abstract.	16
Y	NATUK et al. Adenovirus vectored vaccine. Developmental Biological Standards (1994) Vol. 82, pp. 71-77, see abstract.	1, 9
	·	
•	*	

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Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)			
This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:			
Claim Nos.:  because they relate to subject matter not required to be searched by this Authority, namely:			
Claim Nos.: because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:			
Claim Nos.:     because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).			
Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)			
This International Searching Authority found multiple inventions in this international application, as follows: Please See Continuation Sheet			
<ol> <li>As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.</li> <li>As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.</li> <li>As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:</li> </ol>			
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-5, 8-11, 13-18, 29-32, 34, 35, 37			
Remark on Protest  The additional search fees were accompanied by the applicant's protest.  No protest accompanied the payment of additional search fees.			

Internations	d applica	ation	No.

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# INTERNATIONAL SEARCH REPORT

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING
This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees must be paid.

Group	Claims	
1	1-5, 8-11, 13-18, 29, 30, 31, 32, 34, 35, 37	The claims are directed to an adenoviral vector that is at least partially deleted of <a href="MEL"><u>AEI</u></a> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29) inserted in the parallel orientation of E1. In addition the vector contains a promoter and a polyadenylation signal.
2	6, 7, 36	The claims are directed to an adenoviral vector that is at least partially deleted of <u>AE1</u> and <u>AE3</u> , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Gag protein (SEQ ID NO: 29).
3	12, 33	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV protein inserted in the antiparallel orientation of E1.
4	19-23, 38-42	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Gag protein.
5	24, 27, 28, 43, 46, 47	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle.
6	25, 26, 44, 45	The claim is directed to a method of generating a cellular mediated immune response to HIV Gag protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
7	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the parallel orientation of E1.
8	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the parallel orientation of E1.
9	48-51, 53, 54, 56	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the parallel orientation of E1.
10	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in the antiparallel orientation of E1.
11	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5) inserted in the antiparallel orientation of E1.
12	52	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in the antiparallel orientation of E1.
13	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$



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## INTERNATIONAL SEARCH REPORT

		and ΔΕ3, the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 1) inserted in E1.
14	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 5)
		inserted in E1.
15	55	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Pol protein (SEQ ID NO: 7) inserted in E1.
16	57-61	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Pol protein.
17	62, 65, 66	The claim is directed to a method of generating a cellular mediated immune response
18	63, 64	to HIV Pol protein with the recombinant adenoviral particle.  The claim is directed to a method of generating a cellular mediated immune response
10	05, 04	to HIV Pol protein with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
19	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
-	10.70 -	inserted in the parallel orientation of E1.
20	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type
	73, 75	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
		inserted in the parallel orientation of E1.
21	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	ΔE1, the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13)
		inserted in the parallel orientation of E1.
22	67-70, 72,	The claims are directed to an adenoviral vector that is at least partially deleted of
	73, 75	$\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type
		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15)
		inserted in the parallel orientation of E1.
23	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9) inserted in
	1	the antiparallel orientation of E1.
24	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ ,
	1	the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in
		the antiparallel orientation of E1.
25	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ ,
		the vector contains the cis-acting packaging sequence of the wild type adenovirus
		genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in
	1	the antiparallel orientation of E1.
26	71	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus
	1	genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in
		the antiparallel orientation of E1.
27	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
	1	and AE3, the vector contains the cis-acting packaging sequence of the wild type
	1	adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 9)
		inserted in E1.
28	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$
20	1	and ΔE3, the vector contains the cis-acting packaging sequence of the wild type
20	ł	
20		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11)
29	74	<ul> <li>adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 11) inserted in Ε1.</li> <li>The claim is directed to an adenoviral vector that is at least partially deleted of ΔΕ1</li> </ul>



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		adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 13) inserted in E1.
30	74	The claim is directed to an adenoviral vector that is at least partially deleted of $\Delta E1$ and $\Delta E3$ , the vector contains the cis-acting packaging sequence of the wild type adenovirus genome, and a gene which encodes an HIV Nef protein (SEQ ID NO: 15) inserted in E1.
31	76-80	The claims are directed to a method of making and harvesting of a recombinant adenoviral particle that contains a gene encoding an HIV Nef protein.
32	81, 84, 85	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle.
33	82, 83	The claims are directed to a method of generating a cellular mediated immune response to HIV Nef with the recombinant adenoviral particle in addition to administering a DNA plasmid vaccine.
34	86a	The claim is drawn to a multivalent vaccine wherein gag, pol and nef are expressed from three individual vectors.
35	86b, 88, 89	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from one individual vectors.
36	86c, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-pol fusion and one expressing gag.
37	86d, 87, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing gag-pol fusion and one expressing nef.
38	86e, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from two individual vectors, one expressing nef-gag fusion and one expressing pol.
39	86f, 88	The claims are drawn to a multivalent vaccine wherein gag, pol and nef are expressed from a single vectors as a fusion protein.
40	86g, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed from two individual vectors.
41	86h, 88, 89	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed individually from one vector.
42	86i, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed from two individual vectors.
43	86j, 88, 89	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed from individually from one vector.
44	86k, 88	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually from one vector.
45	861, 88, 89	The claims are drawn to a multivalent vaccine wherein nef and gag are expressed individually from one vector.
46	86m, 88	The claims are drawn to a multivalent vaccine wherein gag and pol are expressed as a fusion protein from one vector.
47	86n, 88	The claims are drawn to a multivalent vaccine wherein pol and nef are expressed as a fusion protein from one vector.
		The claims are drawn to a multivalent vaccine wherein nef and gag are expressed as a

The inventions listed as Groups 1-48 do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

The technical feature linking groups 1-33 appears to be a recombinant adenoviral vector wherein the adenoviral vector is at least partially deleted in E1 but the vector may contain more deletions, the vector contains wild type sequences including packaging signals and a gene encoding a heterologous HIV protein or fragments thereof. Ertl et al. (WO 96/39178) disclose a recombinant adenoviral vector that is deleted in E1 and partially deleted in E3, the remainder of the adenoviral vector contains wild type sequences. The vector additionally contains an insertion of a heterologous protein which includes HIV proteins (see abstract and claims 1 and 5). Therefore, the technical feature linking the inventions of groups 1-45 does not constitute a special technical feature as defined by PCT Rule 13.2, as it does not define a contribution over the prior art.

The special technical feature of the following groups 1-3, 7-15, 19-30 and 34-48 is considered to be the combination of sequences that is disclosed in each group, see individual claim groupings above for the different sequences. The DNA disclosed in each group is made up of a different sequence having a different structure and different function.

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The special technical feature of group 4, 16 and 31 is considered to be a method of producing recombinant adenoviral particles. Each group contains different sequences hence the resulting particles would have different structures and functions associated with the particle.

The special technical feature of group 5, 17 and 32 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors. Each group contains different sequences a encoding different protein, therefore the resulting immune response will also be different.

The special technical feature of group 6, 18 and 33 is considered to be a method of producing a cellular mediated immune response to the heterologous protein encoded by the different adenoviral vectors in conjunction with immunizing the individual a DNA plasmid vaccine. Each method contains different sequences encoding a different protein, therefore the resulting immune response will also be

Accordingly, groups 1-48 are not so linked by the same or corresponding technical feature as to form a single general inventive

Continuation of B. FIELDS SEARCHED Item 3: WEST 2.0, STN-BIOSIS, MEDLINE adenoviral vector, deletion, HIV, Gag, polyadenylation signal, CMV promoter

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